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Greenford, Middlesex UB6 0NN, Great Britain

Patents ADP number *(if you know it)*

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Antibodies

Field of the Invention

The present invention relates to antibodies that bind to NOGO and neutralise the function thereof, polynucleotides encoding such antibodies, pharmaceutical formulations containing said antibodies and to the use of such antibodies in the treatment and/or prophylaxis of neurological diseases. Other aspects, objects and advantages of the present invention will become apparent from the description below.

Background of the Invention

Stroke is a major cause of death and disability in the Western World. There is no approved therapy for the treatment of stroke other than t-PA which has to be administered within 3h of onset following a CT scan to exclude haemorrhage. To date most therapeutic agents directed towards the treatment of acute stroke (i.e. neuroprotection), have predominantly involved targeting glutamate receptors and their down stream signalling pathways known to be involved in acute cell death. However to date these strategies have proved unsuccessful in clinical trials and are often associated with dose-limiting side effects (Hill & Hachinski, The Lancet, 352 : (suppl III) 10-14 (1998)). Therefore there is a need for novel approaches directed towards the amelioration of cell death following the cessation of blood flow.

Following the onset of stroke, some degree of spontaneous functional recovery is observed in many patients, suggesting that the brain has the (albeit limited) ability to repair and/or remodel following injury. Agents that have the potential to enhance this recovery may therefore allow intervention to be made much later (potentially days) following the onset of cerebral ischaemia. Agents which are able to offer both acute neuroprotection and enhance functional recovery may provide significant advantages over current potential neuroprotective strategies.

The mechanisms underlying functional recovery are currently unknown. The sprouting of injured or non-injured axons has been proposed as one possible mechanism. However, although *in vivo* studies have shown that treatment of spinal cord injury or stroke with neurotrophic factors results in enhanced functional recovery and a degree of axonal sprouting, these do not prove a direct link between the degree of axonal sprouting and extent of functional recovery (Jakeman, et al. 1998, Exp. Neurol. 154 : 170-184, Kawamata et al. 1997, Proc Natl Acad. Sci. USA., 94:8179-8184, Ribotta, et al. 2000, J Neurosci. 20 : 5144-5152). Furthermore, axonal sprouting requires a viable neuron. In diseases such as stroke which is associated with extensive cell

death, enhancement of functional recovery offered by a given agent post stroke may therefore be through mechanisms other than axonal sprouting such as differentiation of endogenous stem cells, activation of redundant pathways, changes in receptor distribution or excitability of neurons or glia (Fawcett & Asher, 1999, Brain Res. Bulletin, 49 : 377-391, Horner & Gage, 2000, Nature 407 963-970).

The limited ability of the central nervous system (CNS) to repair following injury is thought in part to be due to molecules within the CNS environment that have an inhibitory effect on axonal sprouting (neurite outgrowth). CNS myelin is thought to contain inhibitory molecules (Schwab ME and Caroni P (1988) *J. Neurosci.* 8, 2381-2193). Two myelin proteins, myelin-associated glycoprotein (MAG) and NOGO have been cloned and identified as putative inhibitors of neurite outgrowth (Sato S. et al (1989) *Biochem. Biophys. Res. Comm.*163,1473-1480; McKerracher L et al (1994) *Neuron* 13, 805-811; Mukhopadhyay G et al (1994) *Neuron* 13, 757-767; Torigoe K and Lundborg G (1997) *Exp. Neurology* 150, 254-262; Schafer et al (1996) *Neuron* 16, 1107-1113; WO9522344; WO9701352; Prinjha R et al (2000) *Nature* 403, 383-384; Chen MS et al (2000) *Nature* 403, 434-439; GrandPre T et al (2000) *Nature* 403, 439-444; US005250414A; WO200005364A1; WO0031235).

Three forms of human NOGO have been identified: NOGO-A having 1192 amino acid residues (GenBank accession no. AJ251383); NOGO-B, a splice variant which lacks residues 186 to 1004 in the putative extracellular domain (GenBank accession no. AJ251384) and a shorter splice variant, NOGO-C, which also lacks residues 186 to 1004 and also has smaller, alternative amino terminal domain (GenBank accession no. AJ251385) (Prinjha et al (2000) *supra).*

Inhibition of the CNS inhibitory proteins such as NOGO may provide a therapeutic means to ameliorate neuronal damage and promote neuronal repair and growth thereby potentially assisting recovery from neuronal injury such as that sustained in stroke. Examples of such NOGO inhibitors may include small molecules, peptides and antibodies.

Antibodies typically comprise two heavy chains linked together by disulphide bonds and two light chains. Each light chain is linked to a respective heavy chain by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains. Each light chain has a variable domain at one end and a constant domain at its other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The constant domains in the light and heavy chains are not involved directly in binding the antibody to antigen.

The variable domains of each pair of light and heavy chains form the antigen binding site. The variable domains on the light and heavy chains have the same general structure and each domain comprises a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs) often referred to as hypervariable regions. The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs are held in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site. CDRs and framework regions of antibodies may be determined by reference to Kabat et al ("Sequences of proteins of immunological interest" US Dept. of Health and Human Services, US Government Printing Office, 1987).

It has been found that anti-MAG monoclonal antibodies, described (Poltorak et al (1987) *Journal of Cell Biology* 105,1893-1899, DeBellard et al (1996) *Mol. Cell. Neurosci.* 7, 89-101; Tang et al (1997) *Mol. Cell. Neurosci.* 9, 333-346; Torigoe K and Lundborg G (1997) *Exp. Neurology* 150, 254-262) and commercially available (MAB1567 (Chemicon)) when administered either directly into the brain or intravenously following focal cerebral ischaemia in the rat (a model of stroke), provides neuroprotection and enhances functional recovery (PCT/EP03/08749).

Therefore anti-MAG antibodies provide potential therapeutic agents for both acute neuroprotection as well as the promotion of functional recovery following stroke. This antibody is a murine antibody. Although murine antibodies are often used as diagnostic agents their utility as a therapeutic has been proven in only a few cases. Their limited application is in part due to the repeated administration of murine monoclonals to humans usually elicits human immune responses against these molecules. To overcome these intrinsic undesireable properties of murine monoclonals "altered" antibodies designed to incorporate regions of human antibodies have been developed and are well established in the art. For example, a humanised antibody contains complementarity determining regions ("CDR's") of non human origin and the majority of the rest of the structure is derived from a human antibody.

It has also been reported that a murine monoclonal antibody, IN-1, that was raised against NI-220/250, a myelin protein which is a potent inhibitor of neurite growth (and subsequently shown to be fragment of NOGO-A), promotes axonal regeneration (Caroni, P and Schwab, ME (1988) Neuron 1 85-96; Schnell, L and Schwab, ME (1990) Nature 343 269-272; Bregman, BS et al (1995) Nature 378 498-501 and Thallmair, M et al (1998) Nature Neuroscience 1 124-131). It has also been reported that NOGO-A is

the antigen for IN-1 (Chen et al (2000) Nature 403 434-439). Administration of IN-1 Fab fragment or humanised IN-1 to rats that have undergone spinal cord transection, enhanced recovery (Fiedler, M et al (2002) Protein Eng 15 931-941; Brosamle, C et al (2000) J. Neuroscience 20 8061-8068). However to date there is no evidence in the literature to suggest that IN-1, or its humanised form, can bind and inhibit human NOGO-A, a necessary requirement for a monoclonal antibody to be useful in the therapeutic treatment of NOGO-mediated diseases and disorders such as stroke and neurodegenerative diseases in humans.

Therefore it remains a highly desirable goal to isolate and develop a therapeutically useful monoclonal antibody that binds and inhibits the activity of human NOGO. The process of neurodegeneration underlies many neurological diseases/disorders including, but not limited to, acute diseases such as stroke, traumatic brain injury and spinal cord injury as well as chronic diseases including Alzheimer's disease, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Huntington's disease, multiple sclerosis and inclusion body myositis.

Consequently an anti-NOGO monoclonal antibody may be useful in the treatment of these diseases/disorders, by both ameliorating the cell death associated with these diseases/disorders and promoting functional recovery.

All publications, both journal and patent, disclosed in this present specification are expressly and entirely incorporated herein by reference.

Brief Summary of the Invention

The invention provides an antibody or functional fragment thereof which binds to and neutralises NOGO, preferably human NOGO. Such antibody may, for example, comprise one or more CDR's as shown in the tables 1 to 6 which show the CDRs of three such independently isolated antibodies: 2A10/3, 2C4/1 and 15C3/3. The CDR's are identified as described by Kabat (Kabat et al. (1991) "Sequences of proteins of immunological interest"; Fifth Edition; US Department of Health and Human Services; NIH publication No 91-3242). CDRs preferably are as defined by Kabat but following the principles of protein structure and folding as defined by Chothia and Lesk, (Chothia et al., (1989) "Conformations of immunoglobulin hypervariable regions"; Nature 342, p877-883) it will be appreciated that additional residues may also be considered to be part of the antigen binding region and are thus encompassed by the present invention.

Table 1: Antibody 2A10/3 light chain CDRs

CDR	According to Kabat
L1	RSSKSLLYKDGKTYLN (SEQ ID NO:1)
L2	LMSTRAS (SEQ ID NO:2)
L3	QQLVEYPLT (SEQ ID NO:3)

Table 2: Antibody 2A10/3 heavy chain CDRs

CDR	According to Kabat
H1	SYWMH (SEQ ID NO:4)
H2	NINPSNGGTNYNEKFKS (SEQ ID NO:5)
H3	GQGY (SEQ ID NO:6)

Table 3: Antibody 2C4/1 light chain CDRs

CDR	According to Kabat
L1	RSSQSLVHSNGNTYLH (SEQ ID NO:7)
L2	KVSNRFS (SEQ ID NO:8)
L3	SQSTHVPLT (SEQ ID NO:9)

Table 4: Antibody 2C4/1 heavy chain CDRs

CDR	According to Kabat
H1	FSCYAMS (SEQ ID NO:10)
H2	SISDGGSYTYYPDNVKG (SEQ ID NO:11)
H3	ELLFDY (SEQ ID NO:12)

Table 5: Antibody 15C3/3 light chain CDRs

CDR	According to Kabat
L1	RSSKSLLHSNGNTYLY (SEQ ID NO:13)
L2	RMSNLAS (SEQ ID NO:14)
L3	MQHLEYPLT (SEQ ID NO:15)

Table 6: Antibody 15C3/3 heavy chain CDRs

CDR	*According to Kabat*
H1	SYWMN (SEQ ID NO:16)
H2	QIYPGDGDTNYNGKFKG (SEQ ID NO:17)
H3	VRFDY (SEQ ID NO:18)

The present invention also relates to an anti-NOGO antibody which binds to the same epitope on the NOGO polypeptide as an antibody having the CDRs described above. Preferably the epitope is comprised within the region 586 to 785 (NOGO-A amino acid numbering), more preferably the epitope is comprised within the region 586 to 685 or 686 to 785. Competitive inhibition assays are used for mapping of the epitopes on an antigen. Thus there is also provided an anti-NOGO antibody which competitvely inhibits the binding of the antibody having the CDRs described *supra* to NOGO, preferably human NOGO.

Also provided is a chimeric antibody which binds to and neutralises NOGO, preferably human NOGO, comprising CDRs such as those disclosed in tables 1 to 6. Preferably the chimeric antibody comprises mouse and human sequences.

Further, the invention provides a humanised antibody which binds to and neutralises NOGO, preferably human NOGO.

In addition the invention provides an antibody which binds to and neutralises NOGO, preferably human NOGO, which is prepared by CDR grafting and which comprises CDRs such as those disclosed in tables 1 to 6.

In a further aspect, the present invention provides an antibody or functional fragment thereof which comprises a heavy chain variable domain which comprises one or more CDR's selected from CDRH1, CDRH2 and CDRH3 of table 1, preferably comprising at least CDRH3, and/or a light chain variable domain which comprises one or more CDRs selected from CDRL1, CDRL2 and CDRL3 from table 4; an antibody or functional fragment thereof which comprises a heavy chain variable domain which comprises one or more CDR's selected from CDRH1, CDRH2 and CDRH3 of table 2, preferably comprising at least CDRH3, and/or a light chain variable domain which comprises one or more CDRs selected from CDRL1, CDRL2 and CDRL3 from table 5; or an antibody or functional fragment thereof which comprises a heavy chain variable domain which comprises one or more CDR's selected from CDRH1, CDRH2 and CDRH3 of table 3, preferably comprising at least CDRH3, and/or a light chain variable

domain which comprises one or more CDRs selected from CDRL1, CDRL2 and CDRL3 from table 6.

The invention further provides an anti-NOGO antibody or functional fragment thereof which comprises:

a) a heavy chain variable domain (V_H) which comprises in sequence CDRH1, CDRH2 and CDRH3 from table 1,

and /or

b) a light chain variable domain (V_L) which comprises in sequence CDRL1, CDRL2 and CDRL3 from table 4;

an anti-NOGO antibody or functional fragment thereof which comprises:

a) a heavy chain variable domain (V_H) which comprises in sequence CDRH1, CDRH2 and CDRH3 from table 2,

and /or

b) a light chain variable domain (V_L) which comprises in sequence CDRL1, CDRL2 and CDRL3 from table 5; or

an anti-NOGO antibody or functional fragment thereof which comprises:

a) a heavy chain variable domain (V_H) which comprises in sequence CDRH1, CDRH2 and CDRH3 from table 3,

and /or

c) a light chain variable domain (V_L) which comprises in sequence CDRL1, CDRL2 and CDRL3 from table 6.

Preferably the anti-NOGO antibody is a humanised anti-NOGO antibody.

Preferably the humanised anti-NOGO antibody is class 1gG, more preferably 1gG1.

A further aspect of the invention provides a pharmaceutical composition comprising an anti-NOGO antibody of the present invention or functional fragment thereof together with a pharmaceutically acceptable diluent or carrier.

In a further aspect, the present invention provides a method of treatment or prophylaxis of stroke and other neurological diseases, in particular Alheimer's disease, in a human which comprises administering to said human in need thereof an effective amount of an anti-NOGO antibody of the invention or functional fragments thereof.

In another aspect, the invention provides the use of an anti-NOGO antibody of the invention or a functional fragment thereof in the preparation of a medicament for treatment or prophylaxis of stroke and other neurological diseases, in particular Alzheimer's disease.

In a further aspect, the present invention provides a method of inhibiting neurodegeneration and/or promoting functional recovery in a human patient afflicted with, or at risk of developing, a stroke or other neurological disease, in particular Alzheimer's disease, which comprises administering to said human in need thereof an effective amount of an anti-NOGO antibody of the invention or a functional fragment thereof.

In a yet further aspect, the invention provides the use of an anti-NOGO antibody of the invention or a functional fragment thereof in the preparation of a medicament for inhibiting neurodegeneration and/or promoting functional recovery in a human patient afflicted with, or at risk of developing, a stroke and other neurological disease, in particular Alzheimer's disease.

Other aspects and advantages of the present invention are described further in the detailed description and the preferred embodiments thereof.

Description of the Figures

Figure 1 shows the inhibitory effect of the GST-NOGO-A56 fusion protein on neurite outgrowth. The Y axis shows the average neurite length/ neurite (NL/N) in arbitrary units.

Figure 2 shows the blocking effect by the supernatant of the hybridoma 2A10 on the neurite outgrowth inhibitory activity of NOGO-A56 (GST-Nogo5&6). The Y axis is as for figure 1.

Figure 3 shows the blocking effect by the supernatant of the hybridoma 2C4 on the neurite outgrowth inhibitory activity of NOGO-A56 (GST-Nogo5&6). The Y axis is as for figure 1.

Figure 4 shows the blocking effect by the supernatant of the hybridoma15C3 on the neurite outgrowth inhibitory activity of NOGO-A56 (GST-Nogo5&6). The Y axis is as for figure 1.

Figure 5 is the control hybridoma supernatant 12G3 which has no NOGO-A56 blocking activity. The Y axis is as for figure 1.

Figure 6 shows the NOGO-A56-blocking effect of purified 2A10 at 4 concentrations. The Y axis is as for figure 1.

Figure 7 shows that recombinant IN-1 monoclonal antibody does not show any blocking activity towards NOGO-A56 (GST-NOGO5&6). Y axis is as for figure 1.

For figures 1 to 7 the negative control is the GST protein alone.

Figure 8 shows the binding of 2A10, 2C4 and 15C3 monoclonal antibodies to human NOGO-A56. The Y-axis shows the measured OD at 450nm, a quantitative measure of antibody captured in the wells. The X-axis shows the concentration of antibody used (ng/ml) per well at each data point.

Detailed Description of the Invention

Anti-NOGO Antibody

The antibody of the invention is preferably a monoclonal antibody (mAb) and is preferably chimeric, humanised or reshaped. Of these humanised is particularly preferred.

The antibody preferably has the structure of a natural antibody or fragment thereof. The antibody may therefore comprise a complete antibody, a $(Fab^1)_2$ fragment, a Fab fragment, a light chain dimer or a heavy chain dimer. The antibody may be an IgG1, IgG2, IgG3, or IgG4; or IgM; IgA, IgE or IgD or a modified variant thereof. The constant domain of the antibody heavy chain may be selected accordingly. The light chain constant domain may be a kappa or lambda constant domain. Furthermore, the antibody may comprise modifications of all classes eg IgG dimers, Fc mutants that no longer bind Fc receptors or mediate Clq binding (blocking antibodies) . The antibody may also be a chimeric antibody of the type described in WO86/01533 which comprises an antigen binding region and a non-immunoglobulin region. The antigen binding region is an antibody light chain variable domain or heavy chain variable domain. Typically the antigen binding region comprises both light and heavy chain variable domains. The non-immunoglobulin region is fused at its C terminus to the antigen binding region. The non-immunoglobulin region is typically a non-immunoglobullin protein and may be an enzyme, a toxin or protein having known binding specificity. The two regions of this type of chimeric antibody may be connected via a cleavable linker sequence. Immunoadhesins having the CDRs as hereinbefore described are also contemplated in the present invention.

The constant region is selected according to the functionality required. Normally an IgG1 will demonstrate lytic ability through binding to complement and/or will mediate ADCC (antibody dependent cell cytotoxicity). An IgG4 will be preferred if an non-cytototoxic blocking antibody is required. However, IgG4 antibodies can demonstrate instability in production and therefore it may be more preferable to modify the generally more stable IgG1. Suggested modifications are described in EP0307434 preferred modifications include at positions 235 and 237. The invention therefore provides a lytic or a non-lytic form of an antibody according to the invention.

In a preferred aspect the antibody is class IgG, more preferably IgG1.

In preferred forms therefore the antibody of the invention is a full length non-lytic IgG1 antibody having the CDRs described *supra*. In most preferred forms we provide a

full length non-lytic IgG1 antibody having the CDRs of SEQ ID NOs 1 to 6; SEQ ID NOs 7 to 12 or SEQ ID NOs 13 to 18.

In a further aspect, the invention provides polynucleotides encoding the CDRs. For example the invention provides polynucleotides encoding CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3 as disclosed in tables 1 to 6. Preferred polynucleotide sequences are shown below in tables 7 to 12.

Table 7: Antibody 2A10/3 light chain CDRs

CDR	
L1	AGGTCTAGTAAGAGTCTCCTATATAAGGATG GGAAGACATACTTGAAT (SEQ ID NO:19)
L2	TTGATGTCCACCCGTGCATCA (SEQ ID NO:20)
L3	CAACAACTTGTAGAGTATCCGCTCACG (SEQ ID NO:21)

Table 8: Antibody 2A10/3 heavy chain CDRs

CDR	
H1	AGCTACTGGATGCAC (SEQ ID NO:22)
H2	AATATTAATCCTAGCAATGGTGGTACTAACTACAAT GAGAAGTTCAAGAGC (SEQ ID NO:23)
H3	GGACAGGGCTAC (SEQ ID NO:24)

Table 9: Antibody 2C4/1 light chain CDRs

CDR	
L1	AGATCTAGTCAGAGCCTTGTACACAGTAATG GAAACACCTATTTACAT (SEQ ID NO:25)
L2	AAAGTTTCCAACCGATTTTCT (SEQ ID NO:26)
L3	TCTCAGAGTACACATGTTCCG CTCACG (SEQ ID NO:27)

Table 10: Antibody 2C4/1 heavy chain CDRs

CDR	

H1	TTCAGTTGCTATGCCATGTCT (SEQ ID NO:28)
H2	TCCATTAGTGATGGTGGTAGTTACACCTACTATCCA GACAATGTAAAGGGC (SEQ ID NO:29)
H3	GAACTACTTTTTGACTAC (SEQ ID NO:30)

Table 11: Antibody 15C3/3 light chain CDRs

CDR	
L1	AGGTCTAGTAAGAGTCTCCTGCATAGTAATGGCAA CACTTACTTGTAT (SEQ ID NO:31)
L2	CGGATGTCCAACCTTGCCTCA (SEQ ID NO:32)
L3	ATGCAACATCTAGAATATCCGCTCACG (SEQ ID NO:33)

Table 12: Antibody 15C/3 heavy chain CDRs

CDR	
H1	AGCTACTGGATGAAC (SEQ ID NO:34)
H2	CAGATTTATCCTGGAGATGGTGATACTAACTACAAC GGAAAGTTCAAGGGC (SEQ ID NO:35)
H3	GTACGCTTTGACTAT (SEQ ID NO:36)

In a further aspect of the invention, there is provided a polynucleotide encoding a light chain variable region of an anti-NOGO antibody including at least one CDR selected from CDRL1, CDRL2 and CDRL3 in tables 1 to 3, more preferably including all 3 CDRs in table 1 or all 3 CDRs in table 2 or all 3 CDRs in table 3.

In a further aspect of the invention, there is provided a polynucleotide encoding a heavy chain variable region of an anti-NOGO antibody including at least one CDR selected from CDRH1, CDRH2 and CDRH3, more preferably including all 3 CDRs in table 4 or all 3 CDRs in table 5 or all 3 CDRs in table 6.

The invention further provides an anti-NOGO antibody, or functional fragment thereof, that binds to and neutralises NOGO which comprises a heavy chain variable region comprising one of the following amino acid sequences:-

QVQLQQPGTELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGNINPSNGGTNYNEKFK
SKATLTVDKSSSTAYMQLSSLTSEDSAVYYCELGQGYWGQGTTLTVSS
(SEQ ID NO: 37); or

EVQLVESGGGLVKPGGSLKLSCAASGFTFSCYAMSWVRQTPEKRLEWVASISDGGSYTYYPDNVK
GRFTISRDNAKNNLYLQMSHLKSEDTAMYYCAKELLFDYWGQGTTLTVSS
(SEQ ID NO:38); or

QVQLQQSGAELVKPGASVKISCKASGYAFSSYWMNWVKQRPGKGLEWIGQIYPGDGDTNYNGKFK
GKATLTADKSSSTAYMQLSSLTSEDSAVYFCAVRFDYWGQGTTLTVSS
(SEQ ID NO:39).

The invention further provides an anti-NOGO antibody, or functional fragment thereof, that binds to and neutralises NOGO which comprises a light chain variable region comprising one of the following amino acid sequences:-

DIVITQDELSNPVTSGESVSISCRSSKSLLYKDGKTYLNWFLQRPGQSPQLLIYLMSTRASGVSD
RFSGSGSGTDFTLEISRVKAEDVGVYYCQQLVEYPLTFGAGTKLELK
(SEQ ID NO:40)

DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRFSGVPD
RFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPLTFGAGTKLELK
(SEQ ID NO:41).

DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLASGVPD
RFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPLTFGAGTKLELK
(SEQ ID NO:42).

The boxed sequences in SEQ ID Nos 37 to 42 represent the CDR sequences according to Kabat et al *supra*.

In a further aspect of the invention there is provided an anti-NOGO antibody, or functional fragment thereof, which binds to and neutralises NOGO which comprises:
a) a heavy chain variable region of SEQ ID NO:37 together with a light chain variable region comprising the amino acid sequence of SEQ ID NO:40; or
b) a heavy chain variable region of SEQ ID NO:38 together with a light chain variable region comprising the amino acid sequence of SEQ ID NO:41; or
c) a heavy chain variable region of SEQ ID NO:39 together with a light chain variable region comprising the amino acid sequence of SEQ ID NO:42.

In a further aspect of the present invention there is provided an anti-NOGO antibody, or functional fragment thereof, comprising either:

a heavy chain variable fragment comprising SEQ ID NO:37 and a constant part or fragment thereof of a human heavy chain; and
a light chain variable fragment comprising SEQ ID No:40 and a constant part or fragment thereof of a human light chain; or
a heavy chain variable fragment comprising SEQ ID NO:38 and a constant part or fragment thereof of a human heavy chain; and
a light chain variable fragment comprising SEQ ID No:41 and a constant part or fragment thereof of a human light chain; or
a heavy chain variable fragment comprising SEQ ID NO:39 and a constant part or fragment thereof of a human heavy chain; and
a light chain variable fragment comprising SEQ ID No:42 and a constant part or fragment thereof of a human light chain.

In a further aspect, the invention provides polynucleotides encoding the heavy chain variable region comprising the amino acid sequences of SEQ ID NOs 37 to 39 and light chain variable regions comprising the amino acid sequences of SEQ ID NOs 40 to 42.

A preferred polynucleotide sequence encoding the amino acid sequence of SEQ ID NO:37 is

CCAGGTCCAACTGCAGCAGCCTGGGACTGAACTGGTGAAGCCTGGGGCTTCAGTGAAGCTGTCCT
GCAAGGCTTCTGGCTACACCTTCACCAGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAA
GGCCTTGAGTGGATTGGAAATATTAATCCTAGCAATGGTGGTACTAACTACAATGAGAAGTTCAA
GAGCAAGGCCACACTGACTGTAGACAAATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGA
CATCTGAGGACTCTGCGGTCTATTATTGTGAACTGGGACAGGGCTACTGGGGCCAAGGCACCACT
CTCACAGTCTCCTCA
(SEQ ID NO:43)

A preferred polynucleotide sequence encoding the amino acid sequence of SEQ ID NO:38 is:

GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGGGTC
CCTGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTTGCTATGCCA
TGTCTTGGGTTCGCCAGACTCCGGAAAAGAGGCTGGAGTGGGTCGCATCC
ATTAGTGATGGTGGTAGTTACACCTACTATCCAGACAATGTAAAGGGCCG
ATTCACCATCTCCAGAGACAATGCCAAGAACAACCTGTACCTGCAAATGA
GCCATCTGAAGTCTGAGGACACAGCCATGTATTACTGTGCAAAGGAACTA
CTTTTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
(SEQ ID NO:44)

A preferred polynucleotide sequence encoding the amino acid sequence of SEQ ID NO:39 is:

CAGGTTCAGCTGCAGCAGTCTGGGGCTGAGCTGGTGAAGCCTGGGGCCTC
AGTGAAGATTTCCTGCAAAGCTTCTGGCTACGCATTCAGTAGCTACTGGA
TGAACTGGGTGAAGCAGAGGCCTGGAAAGGGTCTTGAGTGGATTGGACAG

ATTTATCCTGGAGATGGTGATACTAACTACAACGGAAAGTTCAAGGGCAA
GGCCACACTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAGCTCA
GCAGCCTGACCTCTGAGGACTCTGCGGTCTATTTCTGTGCAGTACGCTTT
GACTATTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
(SEQ ID NO:45)

A preferred polynucleotide sequence encoding the amino acid sequence of SEQ ID NO:40 is:

GATATTGTGATAACCCAGGATGAACTCTCCAATCCTGTCACTTCTGGAGA
ATCAGTTTCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTATATAAGGATG
GGAAGACATACTTGAATTGGTTTCTGCAGAGACCAGGACAATCTCCTCAG
CTCCTGATCTATTTGATGTCCACCCGTGCATCAGGAGTCTCAGACCGGTT
TAGTGGCAGTGGGTCAGGAACAGATTTCACCCTGGAAATCAGTAGAGTGA
AGGCTGAGGATGTGGGTGTGTATTACTGTCAACAACTTGTAGAGTATCCG
CTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAA
(SEQ ID NO:46)

A preferred polynucleotide sequence encoding the amino acid sequence of SEQ ID NO:41 is:

GATGTTGTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGA
TCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCCTTGTACACAGTAATG
GAAACACCTATTTACATTGGTACCTGCAGAAGCCAGGCCAGTCTCCAAAG
CTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTT
CAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGG
AGGCTGAGGATCTGGGAGTTTATTTCTGCTCTCAGAGTACACATGTTCCG
CTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAA
(SEQ ID NO:47)

A preferred polynucleotide sequence encoding the amino acid sequence of SEQ ID NO:42 is:

GATATTGTGATGACTCAGGCTGCACCCTCTGTACCTGTCACTCCTGGAGA
GTCAGTATCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTGCATAGTAATG
GCAACACTTACTTGTATTGGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAG
CTCCTGATATATCGGATGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTT
CAGTGGCAGTGGGTCAGGAACTGCTTTCACACTGAGAATCAGTAGAGTGG
AGGCTGAGGATGTGGGTGTTTATTACTGTATGCAACATCTAGAATATCCG
CTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAA
(SEQ ID NO:48)

In a preferred aspect the anti-NOGO antibody is selected from 2A10/3, 2C4/1 or 15C3/3, or most preferably the humanised form thereof. Most preferably the anti-NOGO antibody is 2A10/3 or the humanised form thereof.

Anti-NOGO antibody 2A10/3 comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO:37 and a light chain variable region having the amino acid sequence of SEQ ID NO:40.

Anti-NOGO antibody 2C4/1 comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO:38 and a light chain variable region having the amino acid sequence of SEQ ID NO:41.

Anti-NOGO antibody 15C3/3 comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO:39 and a light chain variable region having the amino acid sequence of SEQ ID NO:42.

"NOGO" refers to any NOGO polypeptide, including variant forms. This includes, but is not limited to, NOGO-A having 1192 amino acid residues (GenBank accession no. AJ251383); NOGO-B, a splice variant which lacks residues 186 to 1004 in the putative extracellular domain (GenBank accession no. AJ251384) and a shorter splice variant, NOGO-C, which also lacks residues 186 to 1004 and also has smaller, alternative amino terminal domain (GenBank accession no. AJ251385) (Prinjha et al (2000) *supra)*. All references to "NOGO" hereinbelow is understood to include any and all variant forms of NOGO such as NOGO-A and the splice variants described, unless a specific form is indicated.

"Neutralising" refers to inhibition, either total or partial, of NOGO function including its binding to neurones and inhibition of neurite outgrowth.

"Altered antibody" refers to a protein encoded by an altered immunoglobulin coding region, which may be obtained by expression in a selected host cell. Such altered antibodies include engineered antibodies (e.g., chimeric, reshaped, humanized or vectored antibodies) or antibody fragments lacking all or part of an immunoglobulin constant region, e.g., Fv, Fab, or $F(ab)_2$ and the like.

"Altered immunoglobulin coding region" refers to a nucleic acid sequence encoding altered antibody. When the altered antibody is a CDR-grafted or humanized antibody, the sequences that encode the complementarity determining regions (CDRs) from a non-human immunoglobulin are inserted into a first immunoglobulin partner comprising human variable framework sequences. Optionally, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner.

"First immunoglobulin partner" refers to a nucleic acid sequence encoding a human framework or human immunoglobulin variable region in which the native (or naturally-occurring) CDR-encoding regions are replaced by the CDR-encoding regions of a donor antibody. The human variable region can be an immunoglobulin heavy chain, a light chain (or both chains), an analog or functional fragments thereof. Such CDR regions, located within the variable region of antibodies (immunoglobulins) can be determined by known methods in the art. For example Kabat et al. (Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)) disclose rules for locating CDRs. In

addition, computer programs are known which are useful for identifying CDR regions/structures.

"Second immunoglobulin partner" refers to another nucleotide sequence encoding a protein or peptide to which the first immunoglobulin partner is fused in frame or by means of an optional conventional linker sequence (i.e., operatively linked). Preferably it is an immunoglobulin gene. The second immunoglobulin partner may include a nucleic acid sequence encoding the entire constant region for the same (i.e., homologous - the first and second altered antibodies are derived from the same source) or an additional (i.e., heterologous) antibody of interest. It may be an immunoglobulin heavy chain or light chain (or both chains as part of a single polypeptide). The second immunoglobulin partner is not limited to a particular immunoglobulin class or isotype. In addition, the second immunoglobulin partner may comprise part of an immunoglobulin constant region, such as found in a Fab, or $F(ab)_2$ (i.e., a discrete part of an appropriate human constant region or framework region). Such second immunoglobulin partner may also comprise a sequence encoding an integral membrane protein exposed on the outer surface of a host cell, e.g., as part of a phage display library, or a sequence encoding a protein for analytical or diagnostic detection, e.g., horseradish peroxidase, β-galactosidase, etc.

The terms Fv, Fc, Fd, Fab, or $F(ab)_2$ are used with their standard meanings (see, e.g., Harlow et al., Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, (1988)).

As used herein, an "engineered antibody" describes a type of altered antibody, i.e., a full-length synthetic antibody (e.g., a chimeric, reshaped or humanized antibody as opposed to an antibody fragment) in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody are replaced by analogous parts from one or more donor antibodies which have specificity for the selected epitope. For example, such molecules may include antibodies characterized by a humanized heavy chain associated with an unmodified light chain (or chimeric light chain), or vice versa. Engineered antibodies may also be characterized by alteration of the nucleic acid sequences encoding the acceptor antibody light and/or heavy variable domain framework regions in order to retain donor antibody binding specificity. These antibodies can comprise replacement of one or more CDRs (preferably all) from the acceptor antibody with CDRs from a donor antibody described herein.

A "chimeric antibody" refers to a type of engineered antibody which contains a naturally-occurring variable region (light chain and heavy chains) derived from a donor

antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al., Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technology, 9:421 (1991)). A suitable human acceptor antibody may be one selected from a conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody. The prior art describes several ways of producing such humanised antibodies – see for example EP-A-0239400 and EP-A-054951

"Reshaped human antibody" refers to an altered antibody in which minimally at least one CDR from a first human monoclonal donor antibody is substituted for a CDR in a second human acceptor antibody. Preferrably all six CDRs are replaced. More preferrably an entire antigen combining region (e.g., Fv, Fab or $F(ab')_2$) from a first human donor monoclonal antibody is substituted for the corresponding region in a second human acceptor monoclonal antibody. Most preferrably the Fab region from a first human donor is operatively linked to the appropriate constant regions of a second human acceptor antibody to form a full length monoclonal antibody.

A "vectored antibody" refers to an antibody to which an agent has been attached to improve transport through the blood brain barrier (BBB). (Review see Pardridge; Advanced Drug Delivery Review 36, 299-321, 1999). The attachment may be chemical or alternatively the moeity can be engineered into the antibody. One example is to make a chimera with an antibody directed towards a brain capilliary endothelial cell receptor eg an anti-insulin receptor antibody or anti-transferrin receptor antibody (Saito et al (1995) *Proc. Natl. Acad. Sci. USA* 92 10227-31; Pardridge et al (1995) *Pharm. Res.* 12 807-816; Broadwell et al (1996) *Exp. Neurol.* 142 47-65; Bickel et al (1993) *Proc Natl. Acad. Sci. USA* 90, 2618-2622; Friden et al (1996) *J. Pharm. Exp. Ther.* 278 1491-1498,

US5182107, US5154924, US5833988, US5527527). Once bound to the receptor, both components of the bispecific antibody pass across the BBB by the process of transcytosis. Alternatively the agent may be a ligand which binds such cell surface receptors eg insulin, transferrin or low density lipoprotein (Descamps et al (1996) *Am. J. Physiol.* 270 H1149-H1158; Duffy et al (1987) *Brain Res.* 420 32-38; Dehouck et al (1997) *J. Cell Biol.* 1997 877-889). Naturally occuring peptides such as penetratin and SynB1 and Syn B3 which are known to improve transport across the BBB can also be used (Rouselle et al (2000) *Mol. Pharm.*57, 679-686 and Rouselle et al (2001) *Journal of Pharmacology and Experimental Therapeutics*296,124-131).

The term "donor antibody" refers to an antibody (monoclonal, or recombinant) which contributes the amino acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first immunoglobulin partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody.

The term "acceptor antibody" refers to an antibody (monoclonal, or recombinant) heterologous to the donor antibody, which contributes all (or any portion, but preferably all) of the amino acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. Preferably a human antibody is the acceptor antibody.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate). The structure and protein folding of the antibody may mean that other residues are considered part of the antigen binding region and would be understood to be so by a skilled person. See for example Chothia et al., (1989) Conformations of immunoglobulin hypervariable regions; Nature 342, p877-883. For convenience the CDR's as defined by Kabat in SEQ ID Nos 37-42 are boxed.

CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include analogs of the naturally

occurring CDRs, which analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they were derived.

A "functional fragment" is a partial heavy or light chain variable sequence (e.g., minor deletions at the amino or carboxy terminus of the immunoglobulin variable region) which retains the same antigen binding specificity and/or neutralizing ability as the antibody from which the fragment was derived.

An "analog" is an amino acid sequence modified by at least one amino acid, wherein said modification can be chemical or a substitution or a rearrangement of a few amino acids (i.e., no more than 10), which modification permits the amino acid sequence to retain the biological characteristics, e.g., antigen specificity and high affinity, of the unmodified sequence. For example, (silent) mutations can be constructed, via substitutions, when certain endonuclease restriction sites are created within or surrounding CDR-encoding regions. The present invention contemplates the use of analogs of the antibody of the invention. It is well known that minor changes in amino acid or nucleic acid sequences may lead eg to an allelic form of the original protein which retains substantially similar properties. Thus analogs of the antibody of the invention includes those in which the CDRs in the hypervariable region of the heavy and light chains are at least 80% homologous, preferably at least 90 % homologous and more preferably at least 95 % homologous to the CDRs as defined above as CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3 in tables 1 to 6 and retain NOGO neutralising activity. Amino acid sequences are are at least 80% homologous if they have 80% identical amino acid residues in a like position when the sequences are aligned optimally, gaps or insertions being counted as non-identical residues. The invention also contemplates analogs of the antibodies of the invention wherein the framework regions are at least 80%, preferably at least 90% and more preferably at least 95% homologous to the framework regions set forth in SEQ ID NOs 37 to 42. Amino acid sequences are at least 80% homologous if they have 80% identical amino acid residues in a like position when the sequences are aligned optimally, gaps or insertions being counted as non-identical residues.

Analogs may also arise as allelic variations. An "allelic variation or modification" is an alteration in the nucleic acid sequence. Such variations or modifications may be due to degeneracy in the genetic code or may be deliberately engineered to provide desired characteristics. These variations or modifications may or may not result in alterations in any encoded amino acid sequence.

The term "effector agents" refers to non-protein carrier molecules to which the altered antibodies, and/or natural or synthetic light or heavy chains of the donor antibody

or other fragments of the donor antibody may be associated by conventional means. Such non-protein carriers can include conventional carriers used in the diagnostic field, e.g., polystyrene or other plastic beads, polysaccharides, e.g., as used in the BIAcore [Pharmacia] system, or other non-protein substances useful in the medical field and safe for administration to humans and animals. Other effector agents may include a macrocycle, for chelating a heavy metal atom, or radioisotopes. Such effector agents may also be useful to increase the half-life of the altered antibodies, e.g., polyethylene glycol.

Alternatively, one can construct antibodies, altered antibodies and fragments, by immunizing a non-human species (for example, bovine, ovine, monkey, chicken, rodent (e.g., murine and rat), etc.) to generate a desirable immunoglobulin upon presentation with native NOGO from any species against which antibodies cross react with human NOGO can be generated, eg human or chicken. Conventional hybridoma techniques are employed to provide a hybridoma cell line secreting a non-human mAb to NOGO. Such hybridomas are then screened for binding using NOGO coated to 384- or 96-well plates, with biotinylated NOGO bound to a streptavidin coated plate. or in a homogenous europium-APC linked immunoassay using biotinylated NOGO.

A native human antibody can be produced in a human antibody mouse such as the "Xenomouse" (Abgenix) where the mouse immunoglobulin genes have been removed and genes encoding the human immunoglobulins have been inserted into the mouse chromosome. The mice are immunised as normal and develop an antibody reponse that is derived from the human genes. Thus the mouse produces human antibodies obviating the need to humanize the after selection of positive hybridomas. (See Green L.L., *J Immunol Methods* 1999 Dec 10;231(1-2):11-23)

The present invention also includes the use of Fab fragments or $F(ab')_2$ fragments derived from mAbs directed against NOGO. These fragments are useful as agents protective *in vivo*. A Fab fragment contains the entire light chain and amino terminal portion of the heavy chain; and an $F(ab')_2$ fragment is the fragment formed by two Fab fragments bound by disulfide bonds. Fab fragments and $F(ab')_2$ fragments can be obtained by conventional means, e.g., cleavage of mAb with the appropriate proteolytic enzymes, papain and/or pepsin, or by recombinant methods. The Fab and $F(ab')_2$ fragments are useful themselves as therapeutic or prophylactic, and as donors of sequences including the variable regions and CDR sequences useful in the formation of recombinant or humanized antibodies as described herein.

The Fab and F(ab')$_2$ fragments can also be constructed via a combinatorial phage library (see, e.g., Winter et al., Ann. Rev. Immunol., 12:433-455 (1994)) or via immunoglobulin chain shuffling (see, e.g., Marks et al., Bio/Technology, 10:779-783 (1992), which are both hereby incorporated by reference in their entirety.

Thus human antibody fragments (Fv, scFv, Fab) specific for NOGO can be isolated using human antibody fragment phage display libraries. A library of bacteriophage particles, which display the human antibody fragment proteins, are panned against the NOGO protein. Those phage displaying antibody fragments that bind the NOGO are retained from the library and clonally amplified. The human antibody genes are then exicised from the specific bacteriophage and inserted into human IgG expression constructs containing the human IgG constant regions to form the intact human IgG molecule with the variable regions from the isolated bacteriophage specific for NOGO.

The donor antibodies may contribute sequences, such as variable heavy and/or light chain peptide sequences, framework sequences, CDR sequences, functional fragments, and analogs thereof, and the nucleic acid sequences encoding them, useful in designing and obtaining various altered antibodies which are characterized by the antigen binding specificity of the donor antibody.

Taking into account the degeneracy of the genetic code, various coding sequences may be constructed which encode the variable heavy and light chain amino acid sequences, and CDR sequences as well as functional fragments and analogs thereof which share the antigen specificity of the donor antibody. Isolated nucleic acid sequences, or fragments thereof, encoding the variable chain peptide sequences or CDRs can be used to produce altered antibodies, e.g., chimeric or humanized antibodies, or other engineered antibodies when operatively combined with a second immunoglobulin partner.

Altered immunoglobulin molecules can encode altered antibodies which include engineered antibodies such as chimeric antibodies and humanized antibodies. A desired altered immunoglobulin coding region contains CDR-encoding regions that encode peptides having the antigen specificity of an anti-NOGO antibody, preferably a high affinity antibody, inserted into a first immunoglobulin partner (a human framework or human immunoglobulin variable region).

Preferably, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner. The second immunoglobulin partner is defined above, and may include a sequence encoding a second antibody region of interest, for example an Fc region. Second immunoglobulin partners may also include sequences encoding another

immunoglobulin to which the light or heavy chain constant region is fused in frame or by means of a linker sequence. Engineered antibodies directed against functional fragments or analogs of NOGO may be designed to elicit enhanced binding.

The second immunoglobulin partner may also be associated with effector agents as defined above, including non-protein carrier molecules, to which the second immunoglobulin partner may be operatively linked by conventional means.

Fusion or linkage between the second immunoglobulin partners, e.g., antibody sequences, and the effector agent may be by any suitable means, e.g., by conventional covalent or ionic bonds, protein fusions, or hetero-bifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde, and the like. Such techniques are known in the art and readily described in conventional chemistry and biochemistry texts.

Additionally, conventional linker sequences which simply provide for a desired amount of space between the second immunoglobulin partner and the effector agent may also be constructed into the altered immunoglobulin coding region. The design of such linkers is well known to those of skill in the art. In further aspects of the invention we provide diabodies (bivalent or bispecific), triabodies, tetrabodies and other multivalent scFV protein species having one or more CDRs as described *supra* that bind to and neutralise NOGO function.

In still a further embodiment, the antibody of the invention may have attached to it an additional agent. For example, the procedure of recombinant DNA technology may be used to produce an engineered antibody of the invention in which the Fc fragment or CH2-CH3 domain of a complete antibody molecule has been replaced by an enzyme or other detectable molecule (i.e., a polypeptide effector or reporter molecule).

The second immunoglobulin partner may also be operatively linked to a non-immunoglobulin peptide, protein or fragment thereof heterologous to the CDR-containing sequence having the antigen specificity of anti-NOGO antibody. The resulting protein may exhibit both anti-NOGO antigen specificity and characteristics of the non-immunoglobulin upon expression. That fusion partner characteristic may be, e.g., a functional characteristic such as another binding or receptor domain, or a therapeutic characteristic if the fusion partner is itself a therapeutic protein, or additional antigenic characteristics.

Another desirable protein of this invention may comprise a complete antibody molecule, having full length heavy and light chains, or any discrete fragment thereof, such as the Fab or $F(ab')_2$ fragments, a heavy chain dimer, or any minimal recombinant fragments thereof such as an F_V or a single-chain antibody (SCA) or any other molecule

with the same specificity as the selected donor mAb. Such protein may be used in the form of an altered antibody, or may be used in its unfused form.

Whenever the second immunoglobulin partner is derived from an antibody different from the donor antibody, e.g. any isotype or class of immunoglobulin framework or constant regions, an engineered antibody results. Engineered antibodies can comprise immunoglobulin (Ig) constant regions and variable framework regions from one source, e.g., the acceptor antibody, and one or more (preferably all) CDRs from the donor antibody. In addition, alterations, e.g., deletions, substitutions, or additions, of the acceptor mAb light and/or heavy variable domain framework region at the nucleic acid or amino acid levels, or the donor CDR regions may be made in order to retain donor antibody antigen binding specificity.

Such engineered antibodies are designed to employ one (or both) of the variable heavy and/or light chains of the anti-NOGO mAb or one or more of the heavy or light chain CDRs. The engineered antibodies may be neutralising, as above defined.

Such engineered antibodies may include a humanized antibody containing the framework regions of a selected human immunoglobulin or subtype, or a chimeric antibody containing the human heavy and light chain constant regions fused to the anti-NOGO antibody functional fragments. A suitable human (or other animal) acceptor antibody may be one selected from a conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody.

Desirably the heterologous framework and constant regions are selected from human immunoglobulin classes and isotypes, such as IgG (subtypes 1 through 4), IgM, IgA, and IgE. However, the acceptor antibody need not comprise only human immunoglobulin protein sequences. For instance a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding a non-immunoglobulin amino acid sequence such as a polypeptide effector or reporter molecule.

Preferably, in a humanized antibody, the variable domains in both human heavy and light chains have been engineered by one or more CDR replacements. It is possible

to use all six CDRs, or various combinations of less than the six CDRs. Preferably all six CDRs are replaced. It is possible to replace the CDRs only in the human heavy chain, using as light chain the unmodified light chain from the human acceptor antibody. Alternatively, a compatible light chain may be selected from another human antibody by recourse to the conventional antibody databases. The remainder of the engineered antibody may be derived from any suitable acceptor human immunoglobulin.

The engineered humanized antibody thus preferably has the structure of a natural human antibody or a fragment thereof, and possesses the combination of properties required for effective therapeutic use.

It will be understood by those skilled in the art that an engineered antibody may be further modified by changes in variable domain amino acids without necessarily affecting the specificity and high affinity of the donor antibody (i.e., an analog). It is anticipated that heavy and light chain amino acids may be substituted by other amino acids either in the variable domain frameworks or CDRs or both.

In addition, the constant region may be altered to enhance or decrease selective properties of the molecules of the instant invention. For example, dimerization, binding to Fc receptors, or the ability to bind and activate complement (see, e.g., Angal et al., Mol. Immunol, 30:105-108 (1993), Xu et al., J. Biol. Chem, 269:3469-3474 (1994), Winter et al., EP 307,434-B).

An altered antibody which is a chimeric antibody differs from the humanized antibodies described above by providing the entire non-human donor antibody heavy chain and light chain variable regions, including framework regions, in association with immunoglobulin constant regions from other species, preferably human for both chains.

Preferably, the variable light and/or heavy chain sequences and the CDRs of suitable donor mAbs, and their encoding nucleic acid sequences, are utilized in the construction of altered antibodies, preferably humanized antibodies, of this invention, by the following process. The same or similar techniques may also be employed to generate other embodiments of this invention.

A hybridoma producing a selected donor mAb is conventionally cloned, and the DNA of its heavy and light chain variable regions obtained by techniques known to one of skill in the art, e.g., the techniques described in Sambrook *et al.*, (Molecular Cloning (A Laboratory Manual), 2nd edition, Cold Spring Harbor Laboratory (1989)). The variable heavy and light regions containing at least the CDR-encoding regions and those portions of the acceptor mAb light and/or heavy variable domain framework regions required in order to retain donor mAb binding specificity, as well as the remaining immunoglobulin-derived parts of the antibody chain derived from a human

immunoglobulin are obtained using polynucleotide primers and reverse transcriptase. The CDR-encoding regions are identified using a known database and by comparison to other antibodies.

A mouse/human chimeric antibody may then be prepared and assayed for binding ability. Such a chimeric antibody contains the entire non-human donor antibody V_H and V_L regions, in association with human Ig constant regions for both chains.

Homologous framework regions of a heavy chain variable region from a human antibody may be identified using computerized databases, e.g., KABAT®, and a human antibody having homology to the donor antibody will be selected as the acceptor antibody. A suitable light chain variable framework region can be designed in a similar manner.

A humanized antibody may be derived from the chimeric antibody, or preferably, made synthetically by inserting the donor mAb CDR-encoding regions from the heavy and light chains appropriately within the selected heavy and light chain framework. Alternatively, a humanized antibody can be made using standard mutagenesis techniques. Thus, the resulting humanized antibody contains human framework regions and donor mAb CDR-encoding regions. There may be subsequent manipulation of framework residues. The resulting humanized antibody can be expressed in recombinant host cells, e.g., COS, CHO or myeloma cells.

A conventional expression vector or recombinant plasmid is produced by placing these coding sequences for the antibody in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Regulatory sequences include promoter sequences, e.g., CMV promoter, and signal sequences, which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a complementary antibody light or heavy chain. Preferably this second expression vector is identical to the first except insofar as the coding sequences and selectable markers are concerned, so to ensure as far as possible that each polypeptide chain is functionally expressed. Alternatively, the heavy and light chain coding sequences for the altered antibody may reside on a single vector.

A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antibody of the invention. The humanized antibody which includes the association of both the recombinant heavy chain and/or light chain is

screened from culture by appropriate assay, such as ELISA or RIA. Similar conventional techniques may be employed to construct other altered antibodies and molecules.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the conventional pUC series of cloning vectors, may be used. One vector, pUC19, is commercially available from supply houses, such as Amersham (Buckinghamshire, United Kingdom) or Pharmacia (Uppsala, Sweden). Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance), and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

Similarly, the vectors employed for expression of the antibodies may be selected by one of skill in the art from any conventional vector. The vectors also contain selected regulatory sequences (such as CMV promoters) which direct the replication and expression of heterologous DNA sequences in selected host cells. These vectors contain the above described DNA sequences which code for the antibody or altered immunoglobulin coding region. In addition, the vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.

The expression vectors may also be characterized by genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other preferable vector sequences include a poly A signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the antibodies or altered immunoglobulin molecules thereof. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However, most desirably, cells from various strains of *E. coli* are used for replication of the cloning vectors and other steps in the construction of altered antibodies of this invention.

Suitable host cells or cell lines for the expression of the antibody of the invention are preferably mammalian cells such as NS0, Sp2/0, CHO, COS, a fibroblast cell (e.g., 3T3), and myeloma cells, and more preferably a CHO or a myeloma cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook *et al.*, cited above.

Bacterial cells may prove useful as host cells suitable for the expression of the recombinant Fabs of the present invention (see, e.g., Plückthun, A., Immunol. Rev., 130:151-188 (1992)). However, due to the tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form, any recombinant Fab produced in a bacterial cell would have to be screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced in a properly folded form, that bacterial cell would be a desirable host. For example, various strains of *E. coli* used for expression are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Streptomyces*, other bacilli and the like may also be employed in this method.

Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. *Drosophila* and *Lepidoptera* and viral expression systems. See, e.g. Miller *et al.*, Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein.

The general methods by which the vectors may be constructed, the transfection methods required to produce the host cells of the invention, and culture methods necessary to produce the altered antibody of the invention from such host cell are all conventional techniques. Likewise, once produced, the antibodies of the invention may be purified from the cell culture contents according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention. For example, preparation of altered antibodies are described in WO 99/58679 and WO 96/16990.

Yet another method of expression of the antibodies may utilize expression in a transgenic animal, such as described in U. S. Patent No. 4,873,316. This relates to an expression system using the animal's casein promoter which when transgenically incorporated into a mammal permits the female to produce the desired recombinant protein in its milk.

Once expressed by the desired method, the antibody is then examined for *in vitro* activity by use of an appropriate assay. Presently conventional ELISA assay formats are employed to assess qualitative and quantitative binding of the antibody to NOGO. Additionally, other *in vitro* assays may also be used to verify neutralizing efficacy prior to subsequent human clinical studies performed to evaluate the persistence of the antibody in the body despite the usual clearance mechanisms.

The therapeutic agents of this invention may be administered as a prophylactic or post injury, or as otherwise needed. The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient.

The mode of administration of the therapeutic agent of the invention may be any suitable route which delivers the agent to the host. The antagonists and antibodies, and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously, intrathecally, intraperitoneally, intramuscularly, intravenously, or intranasally.

Therapeutic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the antagonist or antibody of the invention as an active ingredient in a pharmaceutically acceptable carrier. In the prophylactic agent of the invention, an aqueous suspension or solution containing the engineered antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the antagonist or antibody of the invention or a cocktail thereof dissolved in an pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.9% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antagonist or antibody of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 30 mg or more preferably, about 5 mg to about

25 mg, of an antagonist or antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 30 and preferably 5 mg to about 25 mg of an engineered antibody of the invention. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania.

It is preferred that the therapeutic agent of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by those of skill in the art. To effectively treat stroke and other neurological diseases in a human, one dose of up to 700 mg per 70 kg body weight of an antagonist or antibody of this invention should be administered parenterally, preferably *i.v.* or i.m. (intramuscularly). Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician.

The antibodies described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

In another aspect, the invention provides a pharmaceutical composition comprising anti-NOGO antibody of the present invention or a functional fragment thereof and a pharmaceutically acceptable carrier for treatment or prophylaxis of stroke and other neurological diseases.

In a yet further aspect, the invention provides a pharmaceutical composition comprising the anti-NOGO antibody of the present invention or a functional fragment thereof and a pharmaceutically acceptable carrier for inhibiting neurodegeneration and/or promoting functional recovery in a human patient suffering, or at risk of developing, a stroke or other neurological disease.

The invention further provides a method of treatment or prophylaxis of stroke and other neurological diseases/disorders, in particular Alzheimer's disease, in a human which comprises administering to said human in need thereof an effective amount of an anti-NOGO antibody or a functional fragment thereof.

Further the invention provides the use of an anti-NOGO antibody, or a functional fragment thereof, in the preparation of a medicament for treatment or prophylaxis of stroke and other neurological diseases/disorders, in particular Alheimer's disease.

The invention also provides a method of inhibiting neurodegeneration and/or promoting functional recovery in a human patient suffering, or at risk of developing, a stroke or other neurological disease/disorder, in particular Alzheimer's disease, which comprises administering to said human in need thereof an effective amount of an anti-NOGO antibody or a functional fragment thereof.

In addition the invention provides the use of an anti-NOGO antibody or a functional fragment thereof in the preparation of a medicament for inhibiting neurodegeneration and/or promoting functional recovery in a human patient afflicted with, or at risk of developing, a stroke and other neurological disease/disorder, in particular Alzheimer's disease.

The invention further provides a method of treating or prophylaxis of stroke or other neurological disease/disorder, in particular Alzheimer's disease, in a human comprising the step of parenteral administration of a therapeutically effective amount of an anti-NOGO antibody. Preferably the anti-NOGO antibody is administered intravenously.

Neurological diseases or disorders as used hereinabove includes, but is not limited to traumatic brain injury, spinal cord, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Huntington's disease, multiple sclerosis and in particular Alzheimer's disease.

The invention also provides a method of promoting axonal sprouting comprising the step of contacting a human axon with an anti-NOGO antibody. This method may be performed in-vitro or in-vivo, preferably the method is performed in-vivo

The following examples illustrate the invention.

Examples

Example 1 – Preparation and selection of the hybridomas

Anti-NOGO monoclonal antibodies are produced by hybridoma cells, the result of the fusion of mouse myeloma cells with B lymphocytes from mice immunised with the target antigen. The hybridoma cell is immortalised by the myeloma fusion partner while the capacity to produce antibodies is provided by the B lymphocyte. Each hybridoma cell makes only one individual antibody with unique specificity hence the term monoclonal.

SJL mice were immunised with 10ug total protein (1:1, human NOGO-A splice (amino acids 186-1004) and rat NOGO-A splice (amino acids 173-975), produced as GST-fusion proteins in E coli BL21) using both CFA and RIBI adjuvants subcutaneously. The mice were then boosted with 5ug of the same proteins using RIBI adjuvant after 4

and 8 days. After a further 3 days, immune cells were harvested from the locally draining lymph nodes and fused with mouse myeloma cells using PEG1500 to generate hybridomas. Individual hybridoma cell lines were cloned by two rounds of limiting dilution.

Initial hybridoma antibody selection was on the basis of direct binding to the NOGO protein(s) on microtitre plates. Subsequently ca. 60 hybridomas were selected based on the ability of soluble protein (consisting of human Nogo-A sequence cleaved from the GST moiety using Precission protease) to compete for this binding activity in ELISA assays.

Example 2 – Cloning of the variable regions

Total RNA was extracted from the selected 2A10/3, 2C4/1 and 15C3/3 hybridoma cells followed by reverse transcription and polymerase chain reaction (RT-PCR) to extract heavy and light variable-domain cDNA sequence. The forward primer for RT-PCR was a mixture of degenerate primers specific for murine immunoglobulin gene leader-sequences and the reverse primer was an isotype-specific antibody directed to the constant regions. PCR primers were designed to carry 5' restriction enzyme recognition sites to enable cloning into pUC19 for DNA sequencing.

RNA extraction

Total RNA was extracted from pellets of 10^6 cells of each hybridoma clone using the SV Total RNA Isolation System from Promega according to manufacturer's instructions.

Reverse transcription

RNA was reverse transcribed to produce cDNA of the variable heavy and light domains using forward primers specific for the murine leader sequences and reverse primers to murine IgGκ constant regions. The IgGγ1 reverse primer was used for hybridomas 2C4/1 and 15C3/3; and the IgGγ2b for 2A10/3. Forward primers carry a SalI restriction enzyme recognition site at the 5' end, with four extra nucleotides added 5'to this for efficient restriction digestion. These primers were adapted from Jones ST and Bendig MM 1991 (Biotechnology 9, 88-89). Reverse primers carry a XmaI restriction enzyme recognition site plus and extra four nucleotides at the 5' ends.

Primers:

Murine V_H leader sequence forward primers:

AG77: 5'-ACT AGT CGA CAT GAA ATG CAG CTG GGT CAT STT CTT C-3'

AG78: 5'-ACT AGT CGA CAT GGG ATG GAG CTR TAT CAT SYT CTT-3'

AG79: 5'-ACT AGT CGA CAT GAA GWT GTG GTT AAA CTG GGT TTT T-3'

AG80: 5'-ACT AGT CGA CAT GRA CTT TGG GYT CAG CTT GRT TT-3'
AG81: 5'-ACT AGT CGA CAT GGA CTC CAG GCT CAA TTT AGT TTT CCT T-3'
AG82: 5'-ACT AGT CGA CAT GGC TGT CYT RGS GCT RCT CTT CTG C-3'
AG83: 5'-ACT AGT CGA CAT GGR ATG GAG CKG GRT CTT TMT CTT-3'
AG84: 5'-ACT AGT CGA CAT GAG AGT GCT GAT TCT TTT GTG-3'
AG85: 5'-ACT AGT CGA CAT GGM TTG GGT GTG GAM CTT GCT ATT CCT G-3'
AG86: 5'-ACT AGT CGA CAT GGG CAG ACT TAC ATT CTC ATT CCT G-3'
AG87: 5'-ACT AGT CGA CAT GGA TTT TGG GCT GAT TTT TTT TAT TG-3'
AG89: 5'-ACT AGT CGA CAT GAT GGT GTT AAG TCT TCT GTA CCT G-3'

Murine V_L leader sequence forward primers:
AG90: 5'-ACT AGT CGA CAT GAA GTT GCC TGT TAG GCT GTT GGT GCT G-3'
AG91: 5'-ACT AGT CGA CAT GGA GWC AGA CAC ACT CCT GYT ATG GGT-3'
AG92: 5'-ACT AGT CGA CAT GAG TGT GCT CAC TCA GGT CCT GGC GTT G-3'
AG93: 5'-ACT AGT CGA CAT GAG GRC CCC TGC TCA GWT TYT TGG MWT CTT G-3'
AG94: 5'-ACT AGT CGA CAT GGA TTT WCA GGT GCA GAT TWT CAG CTT C-3'
AG95: 5'-ACT AGT CGA CAT GAG GTK CYY TGY TSA GYT YCT GRG G-3'
AG96: 5'-ACT AGT CGA CAT GGG CWT CAA GAT GGA GTC ACA KWY YCW GG-3'
AG97: 5'-ACT AGT CGA CAT GTG GGG AYC TKT TTY CMM TTT TTC AAT TG-3'
AG98: 5'-ACT AGT CGA CAT GGT RTC CWC ASC TCA GTT CCT TG-3'
AG99: 5'-ACT AGT CGA CAT GTA TAT ATG TTT GTT GTC TAT TTC T-3'
AG100: 5'-ACT AGT CGA CAT GGA AGC CCC AGC TCA GCT TCT CTT CC-3'
MKV12: 5'-ACT AGT CGA CAT GAA GTT TCC TTC TCA ACT TCT GCT C-3'

Murine γ1 constant region reverse primer:
AG102: 5'-GGA TCC CGG GCC AGT GGA TAG ACA GAT G-3'

Murine γ2b constant region reverse primer:
AG104: 5'-GGA TCC CGG GAG TGG ATA GAC TGA TGG-3'

Murine κ constant region reverse primer:
AG101: 5'-GGA TCC CGG GTG GAT GGT GGG AAG ATG-3'

Pools of murine V_H or V_L leader sequence forward primers were prepared at 50μM. Solutions of the murine γ or κ constant region reverse primers were also prepared at 50μM.

RT-PCR

Reverse transcription of the RNA encoding the variable heavy and light regions was carried out in duplicate using the Access RT-PCR System from Promega according to manufacturer's instructions. Approximately 200ng RNA was included in a 50 μl reaction containing RT-PCR buffer supplied, 0.2 mM dNTPs, 1uM of each primer set, 1uM MgS04 and 5U each of AMV Reverse transcriptase and Tfl DNA polymerase.

RT-PCR cycle:
1- 48°C for 45min
2- 94°C for 2min
3- 94°C for 30sec
4- 50°C for 1min
5- 68°C for 2min
6- 68°C for 7min
steps 3 to 5: repeat 30 times.

pUC19 cloning

The variable region RT-PCR products were purified using a Qiagen MinElute Qiagen PCR Purifcation kit according to their instructions and digested sequentially with XmaI and SalI from New England Biolabs according to manufacture's instructions. They were then loaded on a preparative 1% agarose gel containing 0.5% ethidium bromide and run in TAE buffer at 50mA for 1hour and the V region bands excised under ultra-violet light. The DNA fragments were purified from the gel using the MinElute Gel extraction kit from Qiagen according to manufacturer's instructions. pUC19 vector arms were prepared by digesting pUC19 with SalI and XmaI, then purified using the MinElute Reaction Clean up kit from Qiagen and dephosphorylated using Shrimp alkaline phosphatase (USB) according to the manufacturer's instructions. The concentration of the vector arms and the V-region fragments was estimated from an analytical 1% agarose/ethidium bromide gel, mixed in a molar ratio of 1:2 and ligated using Promega's Quick Ligation kit acording to the manufacturer's instructions.

Ligated plasmids were transformed into DH5a cells (Invitrogen) according manufacturer's instructions. Colonies which grew on L-agar plates containing 100μg/ml ampicillin were selected for DNA sequence analysis.

Variable region sequencing

Colonies were cultured overnight at 37°C in 5ml LB medium supplemented with 100μg/ml ampicillin and plasmid DNA was extracted and purified using the Qiagen QIAprep Spin Miniprep kit according to manufacturer's instructions. The V_H and V_L regions were DNA sequenced using standard M13 forward and reverse primers. The results of the sequencing determination are shown as SEQ ID NOs 43 to 48.

Example 3 – Recombinant anti-NOGO antibodies

Recombinant antibodies having murine 2a/k constant regions could be purified from cells transfected with plasmids comprising the light and heavy variable regions cloned onto mouse IgG2a/k constant region gene segments. The cloned murine V regions were amplified by PCR to introduce restriction sites required for cloning into mammalian expression vectors Rld and Rln. Hind III and Spe I sites were designed in frame with the V_H domain to allow cloning into a modified Rld vector containing the mouse γ2a constant region. Hind III and BsiW I sites were designed in frame the V_L domain and allow cloning into a modified Rln vector containing the mouse κ constant region.

PCR primers

2A10.V_H forward primer:

5'- ACTCATAAGCTTGCCACCATGGGATGGAGCTGTATCATCCTCTTTTTGGTAG -3'

V_H reverse primer:

5'-ACTATGACTAGTGTGCCTTGGCCCCAGTAG-3'

V_L forward primer:

5'- ACTCATAAGCTTGCCACCATGAGGTGCTCTCTTCAGTTTCTG -3'

V_L reverse primer:

5'- ACTATGCGTACGTTTCAGCTCCAGCTTGG -3'

PCR was performed using Hercules (Stratagene) according to the manufacturer's instructions in 50μl volume containing approx 10ng of the pUC19 miniprep containing the V-region, 2% DMSO, 400μM dNTPs, 1μM each primer and buffer supplied. PCR was carried out as follows 1-95 °C 2 mins, 2-95°C 1 min, 3-56 °C 1 min, 4-72°C 1 min. Steps 2-4 30 cycles.

Cloning into expression vectors

The PCR products were purified using the MinElute PCR Purification kit from Qiagen according to manufacturer's instructions. The V_H PCR product and Rld (lgG2a) mammalian expression vector were digested Hind III-Spe I. The V_L PCR product and Rln (k) mammalian expression vector were digested Hind III-BsiW I (NEB) according to manufacturer's instructions. Vectors were ligated to inserts in a 1:2 molar ratio using the Promega Quick Ligation kit. Ligation mixes were transfected into DH5a cells and colonies growing on ampicillin selection were grown up and sent for DNA sequence verification.

Sequencing of recombinant anti-NOGO antibody 2A10/3

The sequence of the 2A10 heavy chain between the HindIII and EcoRI cloning sites was determined to be:

AAGCTTGCCACCATGGGATGGAGCTGTATCATCCTCTTTTTGGTAGCAGC
AGCTACAGGTGTCCACTCCCAGGTCCAACTGCAGCAGCCTGGGACTGAAC
TGGTGAAGCCTGGGGCTTCAGTGAAGCTGTCCTGCAAGGCTTCTGGCTAC
ACCTTCACCAGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGG
CCTTGAGTGGATTGGAAATATTAATCCTAGCAATGGTGGTACTAACTACA
ATGAGAAGTTCAAGAGCAAGGCCACACTGACTGTAGACAAATCCTCCAGC
ACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTA
TTATTGTGAACTGGGACAGGGCTACTGGGGCCAAGGCACACTAGTCACCG
TCTCCTCAGCCAAAACAACAGCCCCATCGGTCTATCCACTGGCCCCTGTG
TGTGGAGATACAACTGGCTCCTCGGTGACTCTAGGATGCCTGGTCAAGGG
TTATTTCCCTGAGCCAGTGACCTTGACCTGGAACTCTGGATCCCTGTCCA
GTGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACCCTC
AGCAGCTCAGTGACTGTAACCTCGAGCACCTGGCCCAGCCAGTCCATCAC
CTGCAATGTGGCCCACCCGGCAAGCAGCACCAAGGTGGACAAGAAAATTG
AGCCCAGAGGGCCCACAATCAAGCCCTGTCCTCCATGCAAATGCCCAGCA
CCTAACCTCCTGGGTGGCCCATCCGTCTTCATCTTCCCTCCAAAGATCAA
GGATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGG
ATGTGAGCGAGGATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAAC
GTGGAAGTACACACAGCTCAGACACAAACCCATAGAGAGGATTACAACAG
TACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCACCAGGACTGGATGA
GTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGACCTCCCAGCGCCC
ATCGAGAGAACCATCTCAAAACCCAAAGGGTCAGTAAGAGCTCCACAGGT
ATATGTCTTGCCTCCACCAGAAGAAGAGATGACTAAGAAACAGGTCACTC
TGACCTGCATGGTCACAGACTTCATGCCTGAAGACATTTACGTGGAGTGG
ACCAACAACGGGAAAACAGAGCTAAACTACAAGAACACTGAACCAGTCCT
GGACTCTGATGGTTCTTACTTCATGTACAGCAAGCTGAGAGTGGAAAAGA
AGAACTGGGTGGAAAGAAATAGCTACTCCTGTTCAGTGGTCCACGAGGGT
CTGCACAATCACCACACGACTAAGAGCTTCTCCCGGACTCCGGGTAAATG
AGAATTC

(SEQ ID NO:49)

The sequence of the 2A10 light chain between the HindIII and EcoRI cloning sites was determined to be:

AAGCTTGCCACCATGAGGTGCTCTCTTCAGTTTCTGGGGGTGCTTATGTT
CTGGATCTCTGGAGTCAGTGGGGATATTGTGATAACCCAGGATGAACTCT

CCAATCCTGTCACTTCTGGAGAATCAGTTTCCATCTCCTGCAGGTCTAGT
AAGAGTCTCCTATATAAGGATGGGAAGACATACTTGAATTGGTTTCTGCA
GAGACCAGGACAATCTCCTCAGCTCCTGATCTATTTGATGTCCACCCGTG
CATCAGGAGTCTCAGACCGGTTTAGTGGCAGTGGGTCAGGAACAGATTTC
ACCCTGGAAATCAGTAGAGTGAAGGCTGAGGATGTGGGTGTGTATTACTG
TCAACAACTTGTAGAGTATCCGCTCACGTTCGGTGCTGGGACCAAGCTGG
AGCTGAAACGTACGGATGCTGCACCGACTGTATCCATCTTCCCACCATCC
AGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAA
CTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAAC
GACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGC
ACCTACAGCATGAGCAGCACCCTCACGTTGACCAAGGACGAGTATGAACG
ACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCA
TTGTCAAGAGCTTCAACAGGAATGAGTGTTAAGAATTC

(SEQ ID NO:50)

Example 3 – Mouse anti-NOGO antibody binds to NOGO

GST-humanNogo-A56 at 5ug/ml in 50mM Tris pH9.5 was coated onto Nunc Immunosorp plates (100ul per well) at 4C overnight. Wells were rinsed once with PBS then incubated with 1% BSA in PBS to block non-specific binding sites at room temperature for 1hour. Antibodies were diluted in PBS to 2ug/ml and 1/3 dilutions made from this. Antibodies were added to wells in triplicate and incubated at 4C overnight. Wells were washed three times with PBS then incubated with Anti-Mouse-HRP (1:1000) for 1hour. Washed five times with PBS and then incubated with 100ul TMB substrate (Sigma) per well for 10minutes. The colour reaction was stopped by the addition of 50ul concentrated HCl. Optical density at 450nm was measured using a plate reader, background values read from wells with no antibody were subtracted.

Figure 7 illustrates the dose-dependent binding of all three anti-Nogo-A monoclonal antibodies, 2A10, 2C4 and 15C3, to human Nogo-A56 in an ELISA assay. The Y-axis shows the measured OD at 450nm, a quantitative measure of antibody captured in the wells. The X-axis shows the concentration of antibody used (ng/ml) per well at each data point. Antibody 2A10 shows the highest signal at a range of concentrations suggestive of a higher affinity for human Nogo-A.

Example 4 – Production of Inhibitory Nogo-A Fragment (NOGO-A56)

A cDNA sequence encoding amino acids 586-785

(MQESLYPAAQLCPSFEESEATPSPVLPDIVMEAPLNSAVPSAGASVIQPSSSPLEASSVNYESIK
HEPENPPPYEEAMSVSLKKVSGIKEEIKEPENINAALQETEAPYISIACDLIKETKLSAEPAPDF
SDYSEMAKVEQPVPDHSELVEDSSPDSEPVDLFSDDSIPDVPQKQDETVMLVKESLTETSFESMI
EYENKE) of human NOGO-A was cloned into the BamHI-XhoI sites of pGEX-6P1 to generate a GST-tagged fusion protein designated Nogo-A56. Plasmid was expressed in BL21 cells in 2XTY medium with 100ug/ml ampicillin following induction with IPTG to

0.5mM at 37C for 3hours. Cell pellets were lysed by sonication and the fusion protein purified using Glutathione-sepharose (Amersham Pharmacia) following manufacturers instructions. Purified protein was eluted using reduced glutathione and extensively dialysed against PBS, quantitated using BSA standards and a BioRad coomassie based protein assay and then stored in aliquots at -80C.

Example 5 - Neurite-outgrowth Assay

Control GST only or GST-NogoA56 fusion proteins were thawed on ice and diluted in 0.5x tissue culture grade PBS to 3pmol/ul. 5ul spots were dried onto the centre of each well of BD-Biocoat poly-d-lysine coated 96 well plates in the tissue-culture cabinet. Once dried, purified antibodies, hybridoma conditioned tissue-culture supernatant or compounds were diluted in HBSS (Life Technologies) and 50ul applied to wells in replicates of between 4 and 8 wells. Control wells of GST alone and GST-Nogo-A56 were treated with HBSS without supplements. After 2hours pretreatment at 37C purified, dissociated cerebellar granule neurons from post-natal day 8 rat brains were added at 20-40,000 neurons per well in a volume of 100ul and incubated at 37C for 24 hours. Cultures were fixed using 4% paraformaldehyde/10% sucrose in PBS for 1 hour then neurites were stained using a polyclonal anti-beta-III-tubulin antibody. Neurite-outgrowth was quantitated using automated image capture and analysis on the Cellomics Arrayscan system.

The results are shown in figures 1 to 6.

Figure 1 shows the inhibitory effect of NOGO-A56 on neurite outgrowth compared with the control protein GST alone.

Figures 1 through 5 show the identification of function-blocking anti-NOGO antibodies 2A10/3, 2C4/1 and 15C3/3 together with a non-function blocking control antibody 12G3. The graphs show the average neurite length in cultures exposed to unpurified antibodies (in supernatants). The data shows the blocking effect of 2A10/3, 2C4/1 and 15C3/3 of the neurite outgrowth inhibitory activity of NOGO-A56. The control is GST alone.

Figure 6 shows the blocking of the neurite outgrowth inhibitory effect of NOGO-A56 by purified 2A10/3.

Example 6 – IN-1 has no blocking activity against human NOGO

The neurite assay as described in example 5, when carried out with the IN-1 antibody, shows that IN-1 does not block the inhibitory activity of human NOGO-A (figure 7).

CLAIMS

1. An antibody or functional fragment thereof which binds to and neutralises human NOGO.

2. An antibody according to claim 1 which binds to a region of human NOGO between 586 to 785 (NOGO-A amino acid numbering).

3. An antibody according to claim 2 which binds to a region of human NOGO between 586 to 685 (NOGO-A amino acid numbering).

4. An antibody according to claim 2 which binds to a region of human NOGO between 686 to 785 (NOGO A amino acid numbering).

5. An antibody according to claim 1 which comprises one or more of the following CDRs:

Light chain CDRs

CDR	***According to Kabat***
L1	RSSKSLLYKDGKTYLN (SEQ ID NO:1)
L2	LMSTRAS (SEQ ID NO:2)
L3	QQLVEYPLT (SEQ ID NO:3)

Heavy chain CDRs

CDR	***According to Kabat***
H1	SYWMH (SEQ ID NO:4)
H2	NINPSNGGTNYNEKFKS (SEQ ID NO:5)
H3	GQGY (SEQ ID NO:6)

6. An antibody according to claim 1 which comprises one or more of the following CDRs:

Light chain CDRs

CDR	According to Kabat
L1	RSSQSLVHSNGNTYLH (SEQ ID NO:7)
L2	KVSNRFS (SEQ ID NO:8)
L3	SQSTHVPLT (SEQ ID NO:9)

Heavy chain CDRs

CDR	According to Kabat
H1	FSCYAMS (SEQ ID NO:10)
H2	SISDGGSYTYYPDNVKG (SEQ ID NO:11)
H3	ELLFDY (SEQ ID NO:12)

7. An antibody according to claim 1 which comprises one or more of the following CDRs:

Light chain CDRs

CDR	According to Kabat
L1	RSSKSLLHSNGNTYLY (SEQ ID NO:13)
L2	RMSNLAS (SEQ ID NO:14)
L3	MQHLEYPLT (SEQ ID NO:15)

Heavy chain CDRs

CDR	According to Kabat
H1	SYWMN (SEQ ID NO:16)
H2	QIYPGDGDTNYNGKFKG (SEQ ID NO:17)
H3	VRFDY (SEQ ID NO:18)

8. An antibody according to claim 5 which comprises a heavy chain variable domain which comprises one or more CDR's selected from CDRH1, CDRH2 and CDRH3 and/or a light chain variable domain which comprises one or more CDRs selected from CDRL1, CDRL2 and CDRL3.

9. An antibody according to claim 6 which comprises a heavy chain variable domain which comprises one or more CDR's selected from CDRH1, CDRH2 and CDRH3 and/or a light chain variable domain which comprises one or more CDRs selected from CDRL1, CDRL2 and CDRL3.

10. An antibody according to claim 7 which comprises a heavy chain variable domain which comprises one or more CDR's selected from CDRH1, CDRH2 and CDRH3 and/or a light chain variable domain which comprises one or more CDRs selected from CDRL1, CDRL2 and CDRL3.

11. An antibody according to claim 8 which comprises a heavy chain variable domain which comprises the CDRs CDRH1, CDRH2 and CDRH3 and a light chain variable domain which comprises the CDRs CDRL1, CDRL2 and CDRL3.

12. An antibody according to claim 9 which comprises a heavy chain variable domain which comprises the CDRs CDRH1, CDRH2 and CDRH3 and a light chain variable domain which comprises the CDRs CDRL1, CDRL2 and CDRL3.

13. An antibody according to claim 10 which comprises a heavy chain variable domain which comprises the CDRs CDRH1, CDRH2 and CDRH3 and a light chain variable domain which comprises the CDRs CDRL1, CDRL2 and CDRL3.

14. An antibody of any one of claims 1 to 13 which is a monoclonal antibody.

15. An antibody of any one of claims 1 to 14 which is a humanised antibody.

16. An antibody according to claim 8 wherein the heavy chain variable region comprises the amino acid sequence:

QVQLQQPGTELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGNINPSNGGTNYNEKFK
SKATLTVDKSSSTAYMQLSSLTSEDSAVYYCELGQGYWGQGTTLTVSS
(SEQ ID NO: 37)

17. An antibody according to claim 9 wherein the heavy chain variable region comprises the amino acid sequence:

EVQLVESGGGLVKPGGSLKLSCAASGFTFSCYAMSWVRQTPEKRLEWVASISDGGSYTYYPDNVK
GRFTISRDNAKNNLYLQMSHLKSEDTAMYYCAKELLFDYWGQGTTLTVSS

(SEQ ID NO:38)

18. An antibody according to claim 10 wherein the heavy chain variable region comprises the amino acid sequence:

QVQLQQSGAELVKPGASVKISCKASGYAFSSYWMNWVKQRPGKGLEWIGQIYPGDGDTNYNGKFK
GKATLTADKSSSTAYMQLSSLTSEDSAVYFCAVRFDYWGQGTTLTVSS
(SEQ ID NO:39)

19. An antibody according to claim 8 wherein the light chain variable region comprises the amino acid sequence:

DIVITQDELSNPVTSGESVSISCRSSKSLLYKDGKTYLNWFLQRPGQSPQLLIYLMSTRASGVSD
RFSGSGSGTDFTLEISRVKAEDVGVYYCQQLVEYPLTFGAGTKLELK
(SEQ ID NO:40)

20. An antibody according to claim 9 wherein the light chain variable region comprises the amino acid sequence:

DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRFSGVPD
RFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPLTFGAGTKLELK
(SEQ ID NO:41).

21. An antibody according to claim 10 wherein the light chain variable region comprises the amino acid sequence:

DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLASGVPD
RFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPLTFGAGTKLELK
(SEQ ID NO:42).

22. An antibody, or functional fragment thereof, according to claim 8 which comprises a heavy chain variable region comprising the following amino acid sequence:

QVQLQQPGTELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGNINPSNGGTNYNEKFK
SKATLTVDKSSSTAYMQLSSLTSEDSAVYYCELGQGYWGQGTTLTVSS
(SEQ ID NO: 37)

and a light chain variable region comprising the following amino acid sequence:

DIVITQDELSNPVTSGESVSISCRSSKSLLYKDGKTYLNWFLQRPGQSPQLLIYLMSTRASGVSD
RFSGSGSGTDFTLEISRVKAEDVGVYYCQQLVEYPLTFGAGTKLELK
(SEQ ID NO:40)

23. An antibody, or functional fragment thereof, according to claim 9 which comprises a heavy chain variable region comprising the following amino acid sequence:

EVQLVESGGGLVKPGGSLKLSCAASGFTFSCYAMSWVRQTPEKRLEWVASISDGGSYTYYPDNVK
GRFTISRDNAKNNLYLQMSHLKSEDTAMYYCAKELLFDYWGQGTTLTVSS
(SEQ ID NO:38)

and a light chain variable region comprising the following amino acid sequence:

DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRFSGVPD
RFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPLTFGAGTKLELK
(SEQ ID NO:41)

24. An antibody, or functional fragment thereof, according to claim 10 which comprises a heavy chain variable region comprising the following amino acid sequence:

QVQLQQSGAELVKPGASVKISCKASGYAFSSYWMNWVKQRPGKGLEWIGQIYPGDGDTNYNGKFK
GKATLTADKSSSTAYMQLSSLTSEDSAVYFCAVRFDYWGQGTTLTVSS
(SEQ ID NO:39)

and a light chain variable region comprising the following amino acid sequence:

DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLASGVPD
RFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPLTFGAGTKLELK
(SEQ ID NO:42).

25. A pharmaceutical composition comprising an anti-NOGO antibody or functional fragment thereof according to any one of claims 1 to 24 together with a pharmaceutically acceptable diluent or carrier.

26. A method of treatment or prophylaxis of stroke and other neurological diseases/disorders in a human which comprises administering to said human in need thereof an effective amount of an anti-NOGO antibody, according to any one of claims 1-24 including altered antibodies or a functional fragment thereof.

27. The use of an anti-NOGO antibody according to any one of claims 1-24, including altered antibodies or a functional fragment thereof in the preparation of a medicament for treatment or prophylaxis of stroke and other neurological diseases/disorders.

28. A method of inhibiting neurodegeneration and/or promoting functional recovery in a human patient suffering, or at risk of developing, a stroke or other neurological disease/disorder which comprises administering to said human in need thereof an effective amount of an anti-NOGO antibody according to any one of claims 1-24, including altered antibodies or a functional fragment thereof.

29. The use of an anti-NOGO antibody according to any one of claims 1-24, including altered antibodies or a functional fragment thereof in the preparation of a medicament for inhibiting neurodegeneration and/or promoting functional recovery in a human patient afflicted with, or at risk of developing, a stroke and other neurological disease/disorder.

30. A method of treating or prophylaxis of stroke or other neurological disease/disorder in a human comprising the step of parenteral administration of a therapeutically effective amount of an anti-NOGO antibody according to any one of claims 1 to 24 to said human.

31. The method of claim 30 wherein the anti-NOGO antibody is administered intravenously.

32. The method of any one of claims 26 to 31 wherein the other neurological disease/disorder is selected from the group consisting of;

traumatic brain injury, spinal cord, Alzheimer's disease, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Huntington's disease and multiple sclerosis.

33. A method of promoting axonal sprouting comprising the step of contacting a human axon with an anti-NOGO antibody of claims 1 to 24.

34. The method of claim 33 wherein the method is *in vitro*.

Abstract

The present invention relates to antibodies to NOGO, pharmaceutical formulations containing them and to the use of such antibodies in the treatment and/or prophylaxis of neurological diseases/disorders

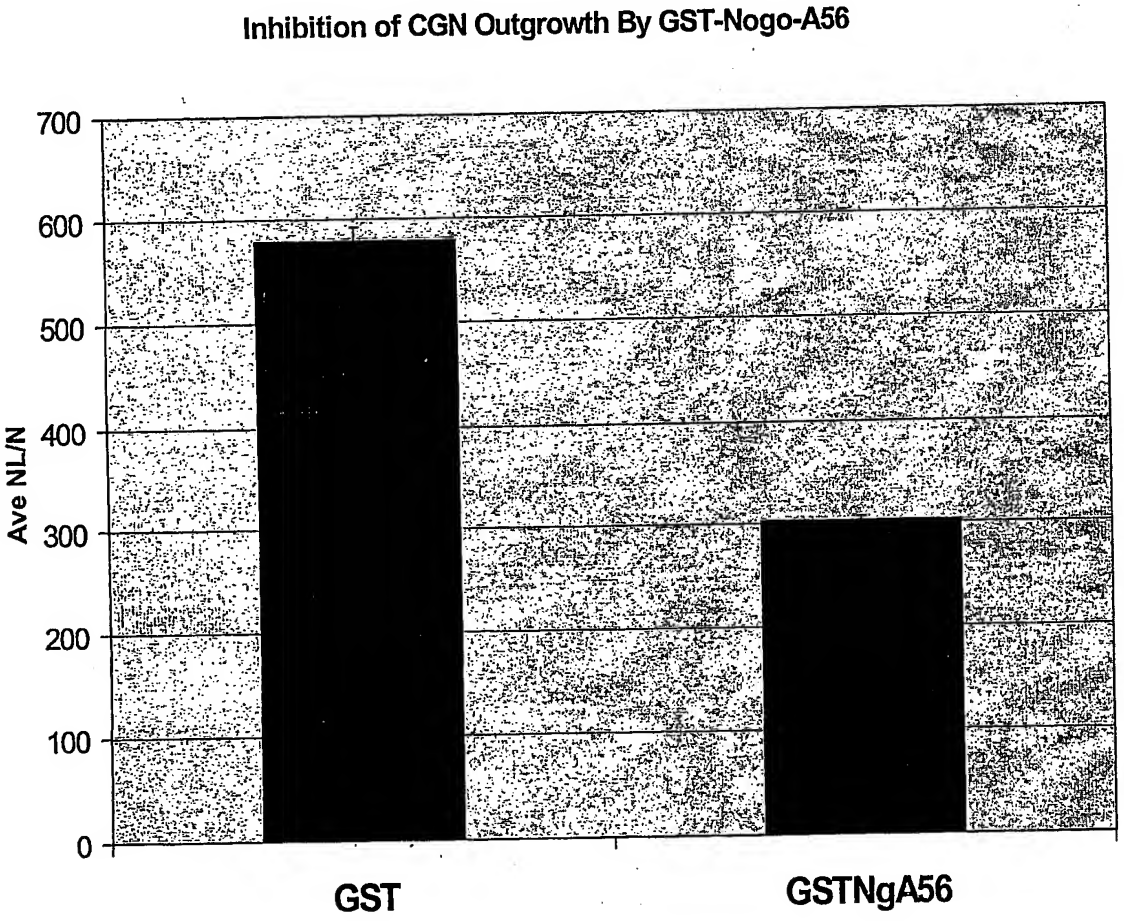
Inhibition of CGN Outgrowth By GST-Nogo-A56
Ave NL/N
700
600
500
400
300
200
100
0
GST
GSTNgA56

Figure 1

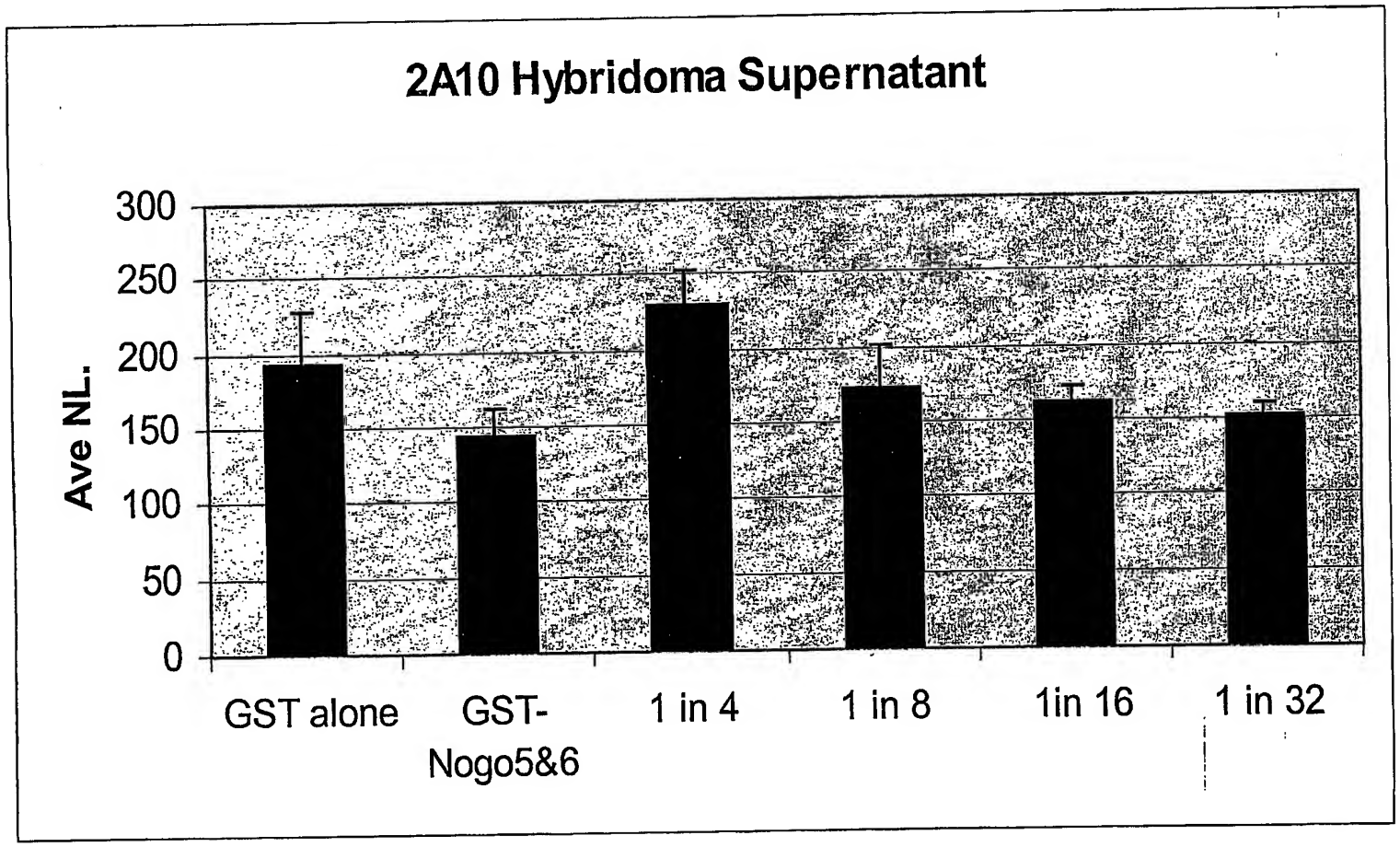
2A10 Hybridoma Supernatant
Ave NL.
300
250
200
150
100
50
0
GST alone
GST-Nogo5&6
1 in 4
1 in 8
1in 16
1 in 32

Figure 2

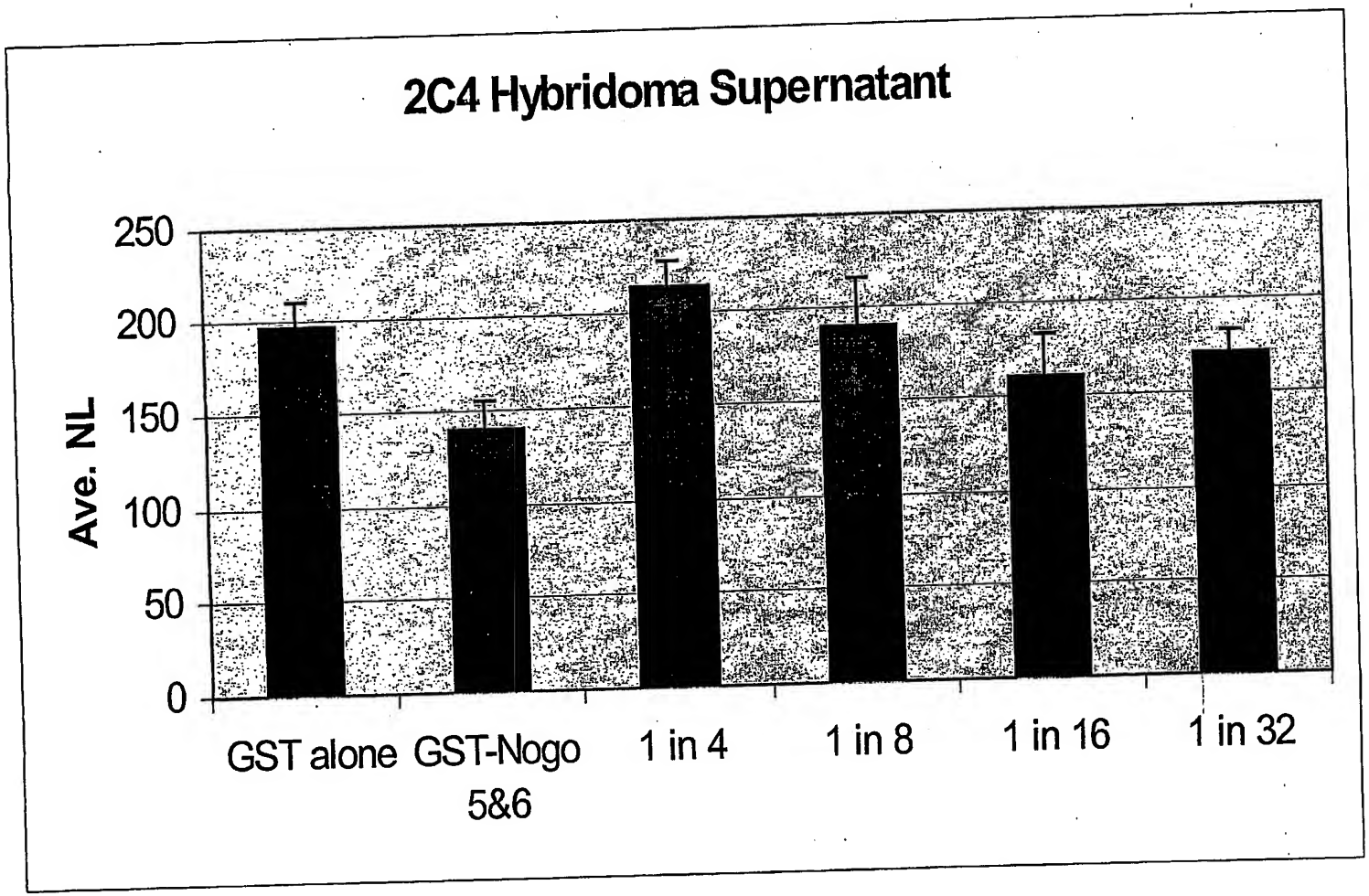
2C4 Hybridoma Supernatant
Ave. NL
250
200
150
100
50
0
GST alone
GST-Nogo 5&6
1 in 4
1 in 8
1 in 16
1 in 32

Figure 3

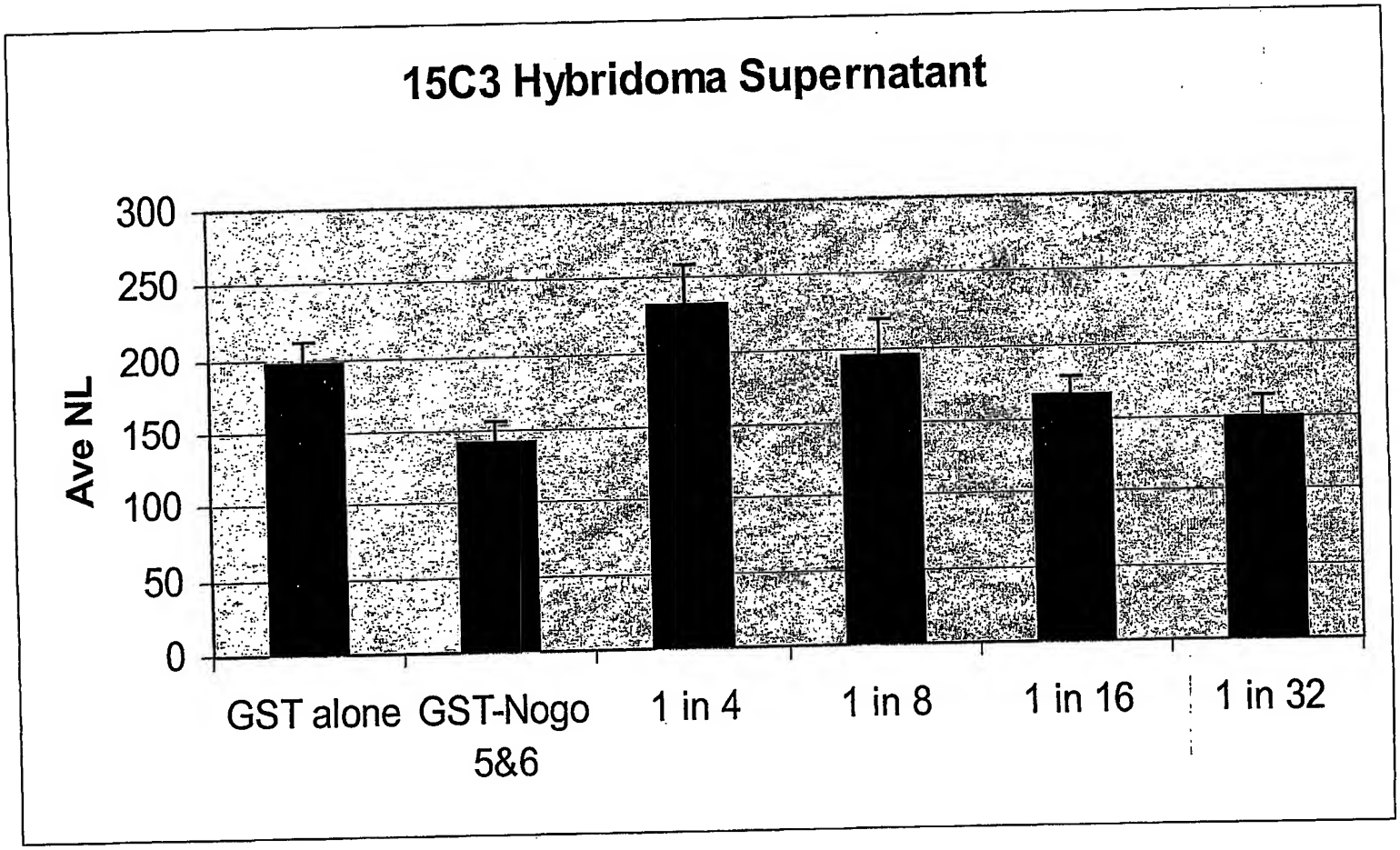
15C3 Hybridoma Supernatant
Ave NL
300
250
200
150
100
50
0
GST alone
GST-Nogo 5&6
1 in 4
1 in 8
1 in 16
1 in 32

Figure 4

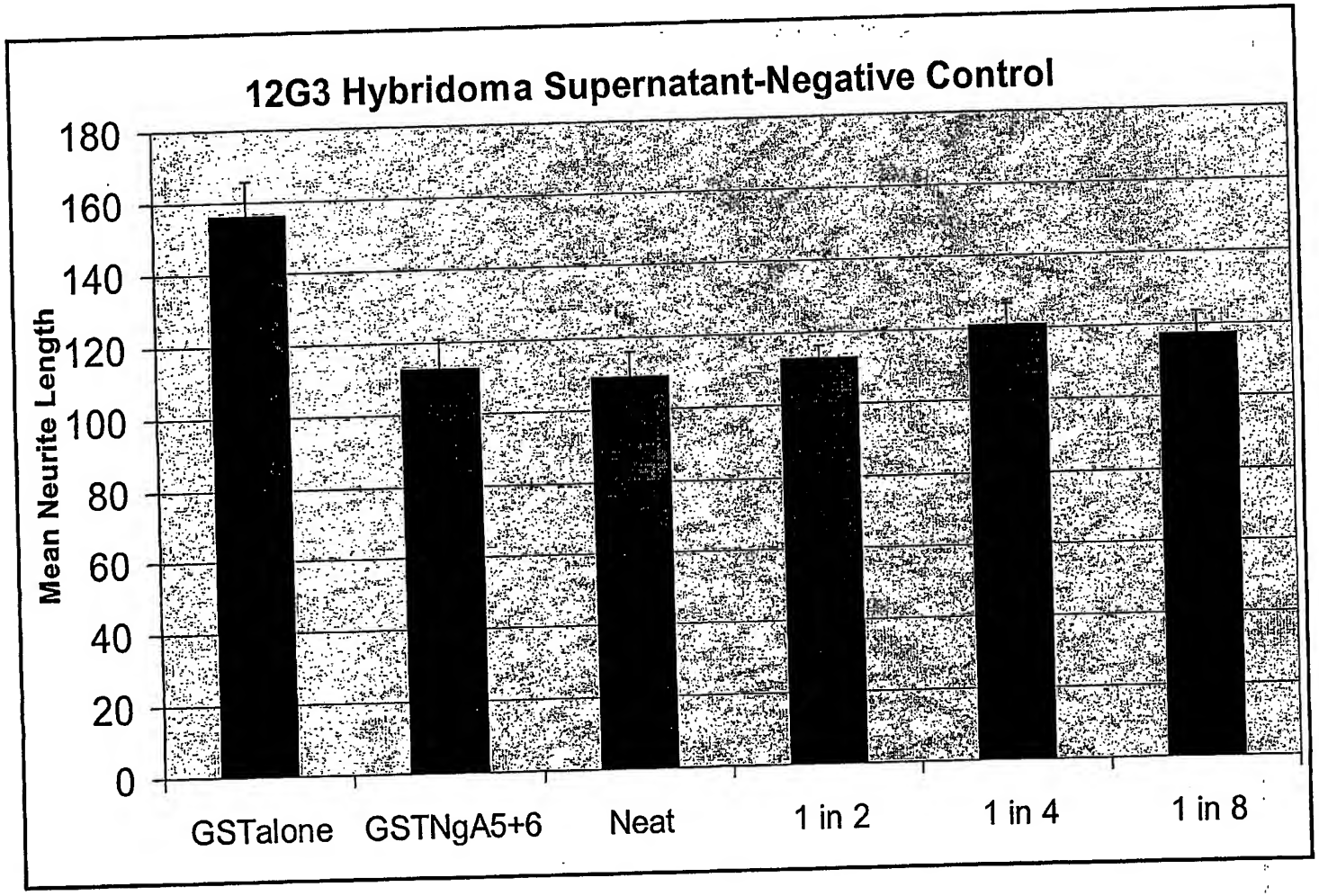
12G3 Hybridoma Supernatant-Negative Control
Mean Neurite Length
180
160
140
120
100
80
60
40
20
0
GSTalone
GSTNgA5+6
Neat
1 in 2
1 in 4
1 in 8

Figure5

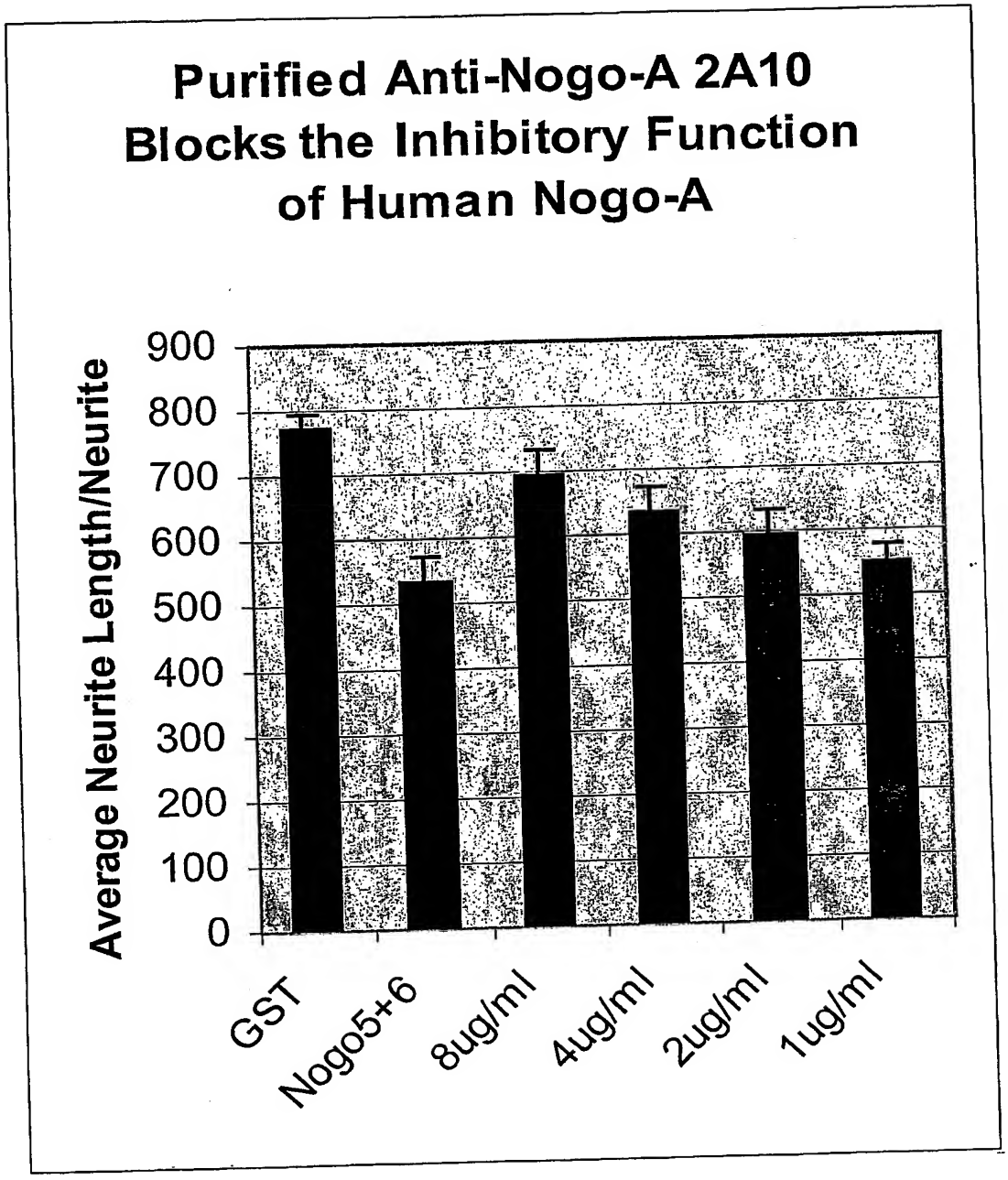
Purified Anti-Nogo-A 2A10
Blocks the Inhibitory Function
of Human Nogo-A
Average Neurite Length/Neurite
900
800
700
600
500
400
300
200
100
0
GST
Nogo5+6
8ug/ml
4ug/ml
2ug/ml
1ug/ml

Figure 6

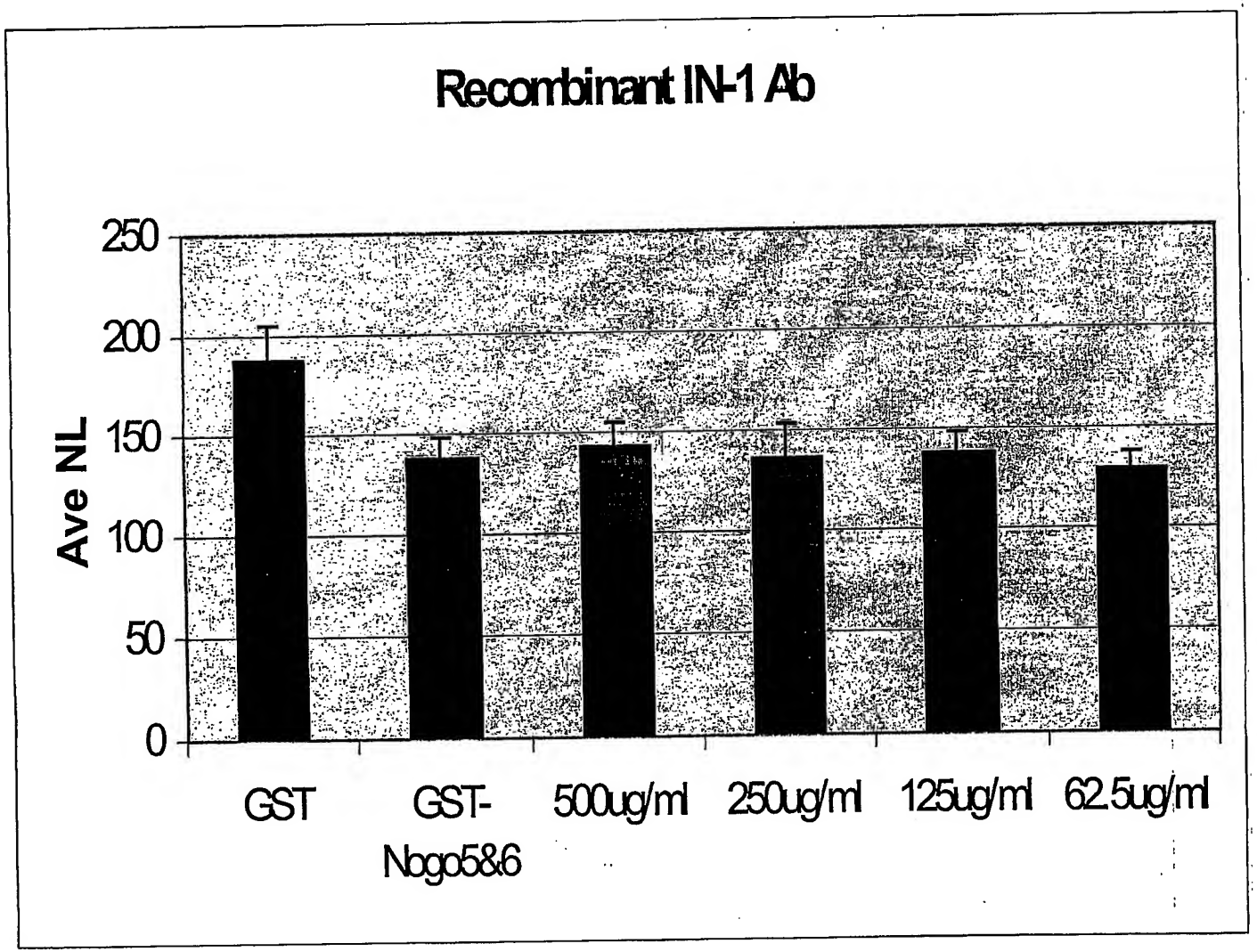
Recombinant IN-1 Ab
Ave NL
250
200
150
100
50
0
GST
GST-Nogo5&6
500ug/ml
250ug/ml
125ug/ml
62.5ug/ml

Figure 7

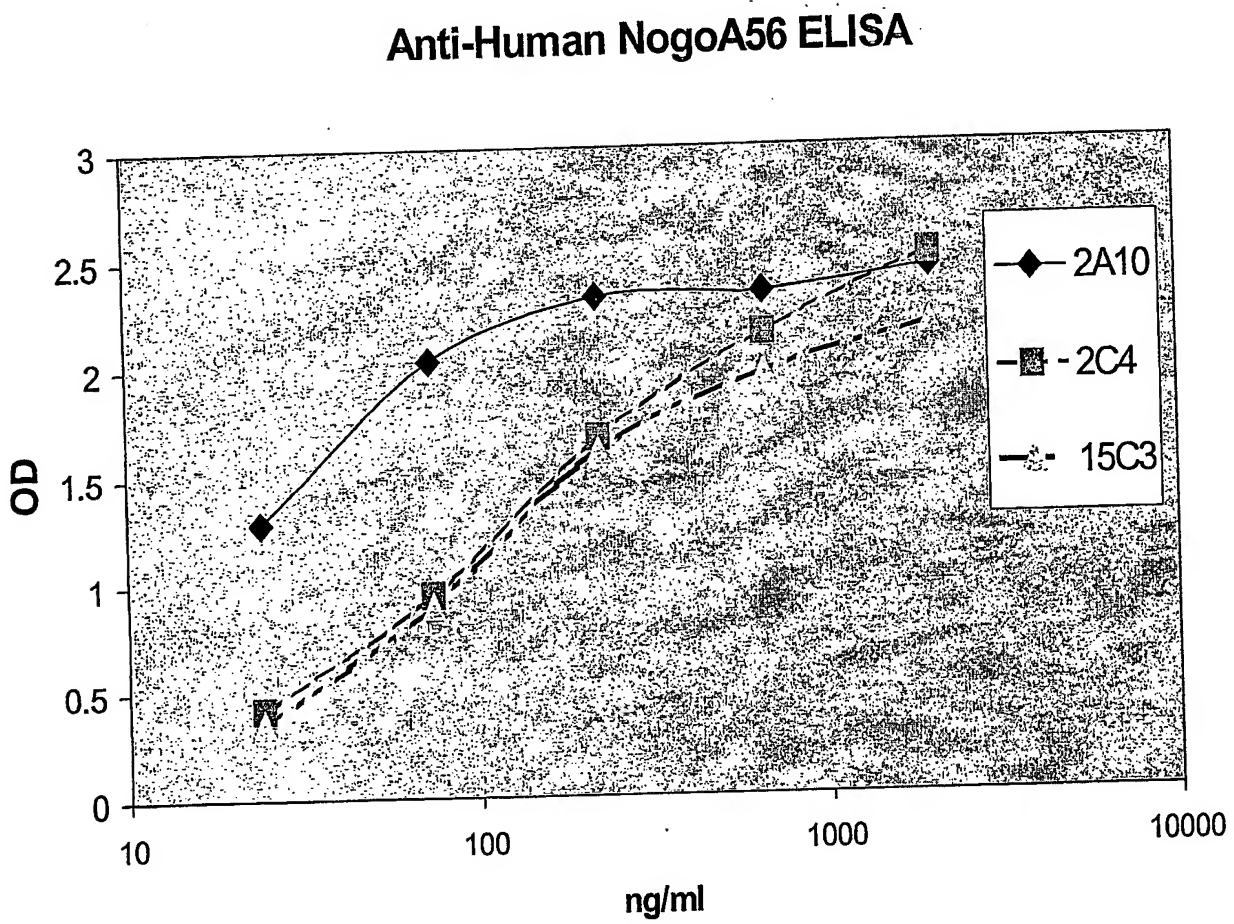
Anti-Human NogoA56 ELISA
OD
3
2.5
2
1.5
1
0.5
0
10
100
1000
10000
ng/ml
2A10
2C4
15C3

Figure 8